ENDOCRINOLOGY

THE BULLETIN OF
THE ASSOCIATION FOR THE STUDY
OF INTERNAL SECRETIONS

VOLUME 22 MARCH, 1938 NUMBER 3

THE THYROTROPIC HORMONE AND THE ANTIHORMONE PROBLEM[1]

SIDNEY C. WERNER

From the Departments of Anatomy and Medicine, College of Physicians and Surgeons, Columbia University, and the Presbyterian Hospital

NEW YORK CITY

Since the initial demonstrations (1, 2, 3) that injections of hypophyseal extracts containing thyrotropic hormone will induce marked activation of the thyroid gland, reports have been fairly uniform in stating that their excessive stimulation of the thyroid gland cannot be maintained much beyond 4 to 5 weeks (4 to 9), except in experiments with the duck (3, 10). The basal metabolic rate of the treated animals has been found to return to normal and the thyroid gland to become microscopically inactive upon continued treatment. Bruner and Starr (11) noted that when the decline in thyroid activity occurred, doses of extract up to 200 times the original did not restimulate the gland. Similar refractoriness has been shown to develop with the other hypophyseal hormones and the gonadotropic principle in pregnancy urine (12 to 15).

Substances inhibitory to the thyrotropic hormone (7, 16) and also to the other hypophyseal principles (17, 18, 19) have been reported in the serum of refractory animals. To explain both the appearance of refractoriness and of an inhibitory substance in the serum, Collip (20) has proposed an antihormone theory, according to which every hormone is conceived to have an antihormone. According to this conception, it is this high titer of antihormone which makes the animals refractory. It has been demonstrated that no antihormone is formed against estrin (D'Amour, 21) so that Collip (22) has limited the applicability of the antihormone theory to the hormones of the hypophysis.

In the opinion of some contributors, neither an antihormone nor an immune response is the correct explanation of refractoriness. Several different theories have been advanced. A readjustment of the endocrine balance has

[1] With the aid of grants administered by Dr. P. E. Smith, from the Committee on Research in Problems of Sex, the National Research Council, and from The Rockefeller Foundation.

been thought by some to be responsible for the phenomenon, occurring as a result of the imbalance produced by the hypophyseal hormones injected (23 to 27). Exhaustion of the stimulated end-organ, e.g., the thyroid, has been proposed by others (8, 28).

The importance of the phenomenon of refractoriness makes investigation important from every angle. In the experiments described in this paper, the loss of responsiveness in chronic treatment with thyrotropic hormone has been found to depend not upon the potency, but on the type of extract used. The work thus supplies additional evidence of the immune nature of refractoriness.

MATERIAL AND METHODS

The thyrotropic effect was determined by measurement of the B.M.R., and the weight, structure and total iodine content of the thyroid gland (10, 29, 30, 31). Dr. G. L. Foster made the iodine determinations, and the author wishes to express his appreciation for this assistance.

Two types of hypophyseal extracts were employed, both of which were made from beef anterior hypophysis, one by the sodium sulphate method of van Dyke and Wallen-Lawrence (32) and the other by the flavianic acid method of Meyer (33).[2] Every batch of extract was assayed on guinea pigs by determining the effect on the B.M.R., and on the weight and structure of the thyroid gland. Physiologically comparable doses of the two types of extract could thus be given.

Two standard strains of male guinea pigs, 250 to 350 gm. in weight, were used. No difference between the two strains in their response to thyrotropic hormone was found. The guinea pigs were kept at a temperature of approximately 25°C. (34), on a diet of oats, lettuce, carrots, hay and water. No iodine was used, or kept in or near the animal room. B.M.R. determinations were made in a closed-system apparatus after a 13 to 16-hour fast. The carbon dioxide output was measured. An R.Q. of 0.81 was assumed and Meeh's formula $S = K\sqrt[3]{w^2}$ was used, in which W = wt. in kg., and $K = 0.085$, which is Rubner's factor for the surface area of guinea pigs. The determinations are given in cc. 0_2/sq.m./min. The animals rested for 1 hour or more in the apparatus before the determinations were made. The animal chambers were submerged in a water bath kept at 30°C. $\pm$ 0.1°C. The temperature within the chambers was thus maintained at the critical temperature for guinea pigs, 32°C. The runs lasted 10 to 25 min., depending on the size and the metabolic rate of the animals. Two runs checking within 5 per cent were usually required for a determination, although an 8 per cent variation between two runs was occasionally included.

Animals were killed the day after the last injection, immediately following the final basal metabolism reading. The thyroid glands were dissected free of fat and fascia, weighed and a small piece fixed in Bouin's fluid for sectioning. The remainder of the gland was dried and used for the determination of its total iodine content. This latter was made by Foster's unpublished modification of Leipert's 1934 method (35).

[2] Meyer's modification of the method published in the monograph of Evans, Meyer and Simpson (25).

CHRONIC TREATMENT WITH BEEF ANTERIOR HYPOPHYSEAL EXTRACTS

Sodium sulphate preparation. Thirteen guinea pigs were injected subcutaneously daily for 23 to 40 days with 1 cc. of the sodium sulphate preparation (tables 1 and 2). The average B.M.R. for the group rose from 109.5 to 152.2 cc. (39 per cent) during the first week of treatment. Two pigs died before the presence of refractoriness could be ascertained. As early as the 28th day, however, the B.M.R. of 7 of the 11 surviving pigs had returned to the normal range, averaging 99.7 cc., and the B.M.R. of 10 of the 11 were within normal range by the 40th day. Autopsy performed on 3 of these 10 refractory animals revealed inactive thyroids microscopically. The glands had refilled with colloid, and the acinar epithelium had returned to a low cuboid type. No gland iodine determinations were made in the group. The loss of response of this group upon prolonged treatment with the sodium sulphate preparation thus confirms the reports in the literature.

TABLE 1. RESULTS OF PROLONGED TREATMENT WITH TWO TYPES OF PITUITARY EXTRACT

Na_2SO_4 Preparation of Beef A.P.			Flavianate Preparation of Beef A.P.		
Days of R	No. of Guinea Pigs		Days of R	No. of Guinea Pigs	
	Activated	Refractory		Activated	Refractory
23–27	1[1]	6	1–27	34	1
28–38	0	3	28–38	5	3
39–40	0	1	39–59	8	3
—	—	—	60–79	7	1
—	—	—	80–88	6	1
Total	1	10	Total	60	9

[1] Gland inactive at autopsy several days later.

Flavianic acid preparation. Sixty-nine guinea pigs were injected subcutaneously every day, for periods ranging up to 88 days, with 0.2 to 0.4 gm. equivalent of dried beef anterior hypophysis as a flavianate (table 1, fig. 1).

Forty-three of these animals were autopsied from 1 to 39 days after the initial injection of the flaviante preparation. In 39, evidence of a high degree of thyroid activation was found. Their B.M.R. averaged 156.0 cc. (128.9 to 195.2 cc.) or 49 per cent above the average oxygen consumption (104.8 cc.) of the controls. The total iodine content of their thyroids had dropped to a low level. This drop in the thyroid iodine of the experimental animals took place during the first 3 days of treatment (6 animals), reaching an average value of 0.019 per cent (0.005 to 0.043 per cent), which was maintained throughout the experiment by the remaining 33 animals. Control figures for the thyroid iodine during this period averaged 0.156 per cent of the dried weight of gland. A lowering of the iodine content was found to have begun 13 hours after a single injection in 2 of 4 animals. Microscopic study likewise confirmed evidence of the marked hyperactivation of the thyroid gland. Sections qualitatively resembled those of the long-term stimulated animals described below, and did not differ from the descriptions in the literature.

Four of the 43 guinea pigs in this short-term treated group, which became

refractory, showed a return of their B.M.R. to normal limits (average 114.2 cc.) by the 39th day of the experiment. Two of these 4 animals were autopsied several weeks after the decline of the B.M.R., and, despite uninterrupted injections, had structurally inactive thyroid glands. The total iodine content of these glands was at the lower limit of normal: 0.067 per cent and 0.088 per cent.

The remaining 26 of the original group of 69 guinea pigs were treated for 39 days or more with the flavianate. The results were similar to those secured in the animals treated with this extract for the shorter periods (table 2). The thyroid glands of 21 of the 26 animals continued to be activated to a

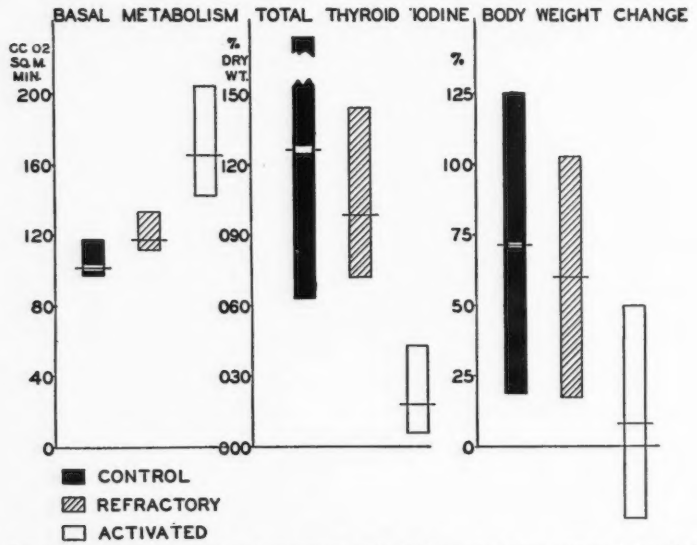

Fig. 1. Graph showing extreme and average values for B.M.R., total iodine content of thyroid gland, and body weight change in guinea pigs treated with beef hypophyseal flavianate.

marked degree, and 5 became refractory. Nineteen of the 26 were autopsied from 30 to 80 days and 7 from 80 to 88 days after the start of injections. Only one of this latter group of 7 became refractory.

The animals whose thyroid glands showed continued activation throughout the long period of injections had an average B.M.R. of 170.9 cc. (range 143.6 to 205.3 cc.). This is 54 per cent higher than the average B.M.R. of the controls during this period. The thyroid gland iodine content averaged only 0.016 per cent of the dried weight of gland for the group (range 0.006 to 0.030 per cent) as opposed to the normal value in the controls of 0.10 per cent. These findings harmonize with those of Schockaert and Foster (10) in the duck. Microscopically, the thyroid glands of these activated animals presented no significant differences from the descriptions in the literature of glands stimulated for shorter periods with hypophyseal extracts (fig. 2). Colloid was almost emptied from the majority of the alveoli, and that which

remained was foamy and pale staining. As reported by others, occasional small cysts, filled with this pale material, were found. The alveolar epithelium was swollen, infolded and hyperplastic, and most of the alveolar spaces were obliterated by the infoldings of the walls of the alveoli.

The 5 animals which became refractory in the long-term group had an average B.M.R. of 120.9, or 10 per cent higher than that of the controls. Their thyroid iodine values averaged 0.098 per cent of dried weight of gland, which is slightly lower than the control figure (0.10 per cent). The persistent slight elevation of their B.M.R. confirms the findings of Siebert and Smith (6), in which partial refractoriness was induced with an acid

TABLE 2. SUMMARY OF EXPERIMENTAL AND CONTROL VALUES IN LONG-TERM FLAVIANATE TREATED GUINEA PIGS

Classification	Days of Treatment	No. of Animals	B.M.R. cc. O_2/sq.m./min.			Total Iodine Content (% dry wt. of Gland)			Body Wt. Change, %			Thyroid Gland Wt. (% Body Wt.)		
			Low	High	Mean	Low	High	Mean	Low	High	Mean	Low	High	Mean
Activated	0- 2	6	139.4	167.3	152.2	.015	.13	.064	—	—	—	.016	.025	.022
	3-38	33	128.9	195.2	156.0	.005	.043	.019	− 50	− 3	− 17	.013	.109	.040
	39-59	8	143.6	182.4	162.9	.006	.025	.014	− 22	+ 50	+ 4	.032	.078	.062
	60-79	6	159.2	184.5	169.8	.010	.030	.018	− 10	+ 41	+ 4	.039	.079	.057
	80-88	7	168.4	205.3	178.6	.013	.019	.016	+ 3	+ 34	+ 17	.042	.078	.059
Summary	39-88	21	143.6	205.3	170.9	.006	.030	.016	− 10	+ 50	+ 8	.032	.079	.059
Refractory	0-38	4	111.7	117.1	114.2	—	—	.088[1]	+ 35	+226	+140			
	39-59	3	117.0	133.0	123.5	.073	.090	.084	+ 18	+104	+ 60	.016	.029	.023
	60-79	1	112.0	113.5	112.8	—	—	.093	+ 78	+ 83	+ 81			.024
	80-88	1	—	—	125.3	—	—	.145	—	—	+ 60	—	—	.019
Summary	39-88	6	112.0	133.0	120.9	.073	.145	.098	+ 18	+104	+ 60	.016	.029	.022
Controls	0-38	14	94.2	117.4	104.8	.100	.290	.153	—	—	—			
	39-59	3	113.4	117.9	115.2	.090	.120	.109	+ 68	+ 83	+ 76	.010	.013	.011
	60-79	1	101.2	101.2	101.2	—	—	.146	—	—	+ 73	—	—	.012
	80-88	3	106.6	114.3	110.5	.063	.112	.082	+ 20	+126	+ 67	.008	.015	.012
Summary	39-88	7	101.2	117.9	111.3	.063	.120	.102	+ 20	+126	+ 72	.008	.015	.011

[1] One case.

extract of anterior hypophysis. However, some of the refractory sodium sulphate extract treated animals showed a B.M.R. lower than normal, as has been reported by Collip and Anderson (7) and Friedgood (8) with similar types of extract. Microscopically, there was a return to the normal appearance for this age (fig. 2*C*). The glands had refilled with colloid, and the epithelium had become a low cuboidal or squamous type. Resorption vacuoles were present in the colloid adjacent to the free cell border.

The refractory animals did not respond to the administration of double the dose of extract, as shown by their B.M.R. Three animals, treated for only 1 to 2 months, however, showed a rise in the B.M.R. with an increased dosage. These animals were not considered refractory and are included in the activated group.

Pneumonia was responsible for a number of deaths in the early part of the work. Four such untreated animals showed no significant difference from the other controls, either in thyroid structure or iodine content, and these have been included in this series. Farrant (36) also observed no thyroid pathology after lobar pneumonia, while Gutman and Foster (31) found the total iodine content of the thyroid unaffected.

In 3 animals not included above, and treated with the flavianate extract

for 46, 60 and 79 days respectively, the B.M.R. and thyroid histology were not in agreement with the iodine content. The B.M.R., measured in only 2 of the 3, was 173.7 and 146.3 cc. respectively. Structurally, the thyroids of all these showed definite activation, but the thyroid iodine varied, being 0.067, 0.085 and 0.161 per cent of the dried weight of the gland. It was felt that these guinea pigs were becoming refractory, but that the return of the gland iodine values to normal had preceded the histological change.

Controls. Twenty-one guinea pigs served as control material (table 1, fig. 1). Fourteen were treated with a flavianate prepared from beef muscle instead of anterior hypophysis, 0.25 gm. equivalent of dried muscle being given daily, except in several instances when doses equivalent to 1.0 gm. were given for periods of a week. The other 7 guinea pigs were kept under identical conditions but not treated, and the controls were killed at intervals up to 80 days. No difference was found between those treated with muscle flavianate and those which received no injections (fig. 1, table 2).

The B.M.R. of the controls kept less than 39 days averaged 104.8 $\pm$ 10 cc., with one extreme value 23.2 cc. above, and one 15.7 cc. below, this average. For the group kept over 39 days, the average B.M.R. was 111.3 cc., ranging from 101.2 to 117.9 cc. The thyroid gland iodine determinations were fairly uniform, averaging 0.153 per cent dried weight of gland for the short-term group. The lower limit was 0.10 per cent but the upper limit was unduly high, due to the presence of one extreme value of 0.29 per cent. For the long-term controls, the average was 0.102 per cent with a range of 0.063 to 0.120 per cent.

Two additional responses were found to be associated with activation, one of which was the weight of the thyroid. This has been extensively used in assays (37). The weight of the thyroids of the animals which continued to show activation after 39 days of treatment averaged 0.059 per cent of their body weight (range, 0.032 to 0.079), whereas the thyroids of those which became refractory either early or late in treatment averaged 0.022 per cent (range .016 to .029) and of the untreated controls .011 per cent (range .008 to .015). The other response of value was the change in body weight. Animals with activated thyroids lost in weight or, at most, gained but little. The onset of refractoriness was accompanied by a rapid weight increase, and the controls gained throughout.

IMMUNOLOGICAL STUDIES WITH TWO TYPES OF BEEF ANTERIOR HYPOPHYSEAL EXTRACT

Effect of immunizing doses. The above findings showed that, with large doses of the two types of hypophyseal extract, there was a marked difference in the production of refractoriness. It therefore seemed desirable to investigate the effect of small non-stimulating doses upon the development of this condition (11, 12). Two groups of 6 guinea pigs each were employed. Five of the first group were given 0.1 cc. of the sodium sulphate preparation subcutaneously every other day for 32 days. The sixth was kept as an untreated control. The B.M.R. averaged 113.1 cc. during the second week of treatment

and 107.2 cc. at the end of the injection period. Thus, during the injection period, no significant change from the control values occurred with the dose used.

All 6 animals were then injected with 20 times the initial dose, i.e., 2 cc.,

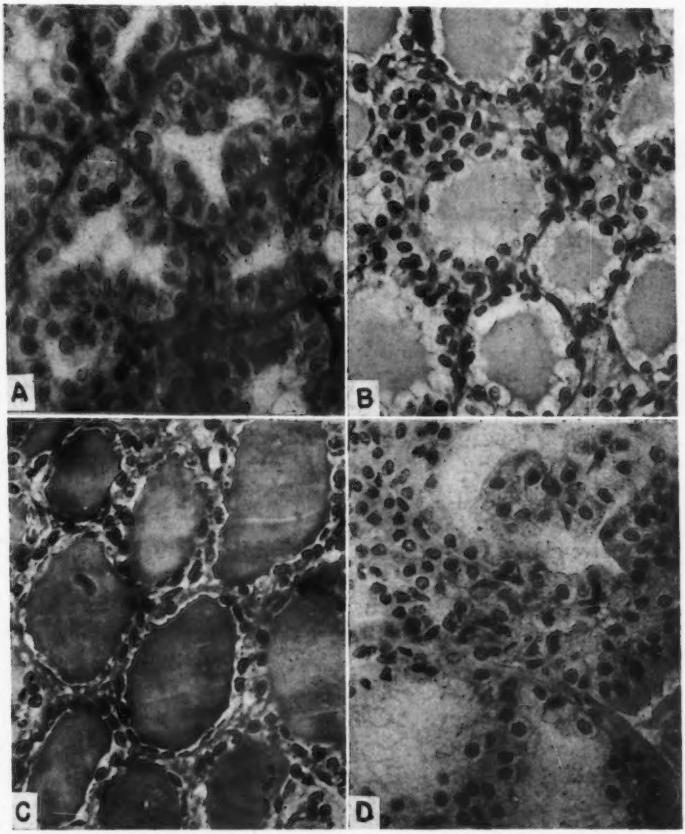

Fig. 2, *A*, Thyroid of *guinea pig 203* after 88 days of treatment with beef hypophyseal flavianate. Intense activation is shown. ×312.
B, Control thyroid gland, 60 days after start of experiment. No treatment. *Guinea pig 234*. ×312.
C, Biopsy of the thyroid gland of *guinea pig 276*, which had become refractory to the sodium sulphate extract of hypophysis. ×312.
D, Thyroid gland of the same guinea pig (276) shown in figure 2*C*, after the substitution of hypophyseal flavianate. for the sodium sulphate preparation. ×312.

subcutaneously daily for 4 days. The control showed a rise in its B.M.R. of 23.6 cc. (21 per cent) on the fifth day. The 5 animals previously given small doses, however, showed no rise, and averaged 111.6 cc. Autopsy specimen of one of the 5 and biopsy specimens of the other 4, microscopically showed inactive thyroid glands, in every instance.

Five of a second group of 6 guinea pigs were treated as above, except

that the flavianate, instead of the Na_2SO_4 preparation, was used. Doses of 0.02 gm. equivalent of dried beef anterior hypophysis were given subcutaneously every other day for 42 days. The sixth guinea pig remained untreated. The average B.M.R. of this group during the second week was 113.1 cc. and, at the end of treatment, 107.7 cc. All 6 animals were then given subcutaneously 20 times this dose, i.e., 0.4 gm. equivalent daily for 4 days. The B.M.R. of the control showed an increase of 21.3 cc. (19 per cent) on the fifth day. The B.M.R. of 3 of the 5 previously treated animals also showed increases of 22.3, 24.3 and 25.6 cc. (19, 24 and 27 per cent), respectively. Autopsy showed that the thyroid glands of these 3 were definitely activated. This activation was somewhat less than that of the control (+++, instead of ++++, the maximum). The other 2 guinea pigs were not stimulated by the large doses of flavianate. Thus, some immunization resulted with small doses of the flavianate preparation, although this was incomplete compared with the immunity resulting from the sodium sulphate preparation.

Stimulation of the thyroids refractory to the Na_2SO_4 extract by injection of the flavianate preparation. Fourteen guinea pigs were made refractory to the sodium sulphate preparation after daily injections of 1 cc. of it for 40 days, except for a period of one week. Ten were then tested for refractoriness by subcutaneous administration of 4 cc. of a half-strength Na_2SO_4 preparation daily for 4 days, and the other 4 with 2 cc. of a full-strength solution. This large dose produced no thyroid response in any of these treated animals, although causing marked activation in previously untreated guinea pigs. Biopsy specimens of the thyroid glands of each of the refractory animals showed inactive glands (fig. 2*C*), and their B.M.R. was within normal limits, averaging 110.8 cc.

Eleven of the 14 were then shifted to injections of the flavianate. A daily dose of 0.4 gm. equivalent of dried beef anterior hypophysis was given subcutaneously to each for 4 days. The B.M.R. of 6 of the 11, measured the day after the last injection, showed a rise of about 26.0 cc., while the other 5 showed no significant change. Histologically, marked activation had taken place in the glands of 9 of the 11 (fig. 2*D*). The 2 which microscopically showed no stimulation likewise exhibited no rise in the B.M.R. The 3 animals not shifted to the flavianate were biopsied and maintained on the sodium sulphate preparation, in order to determine if the biopsy procedure had any effect. No demonstrable renewal of thyroid activity occurred. The B.M.R. of these latter remained as before the biopsy, and sections showed a typically refractory gland. An untreated control was biopsied as above, without inducing any change in thyroid structure.

DISCUSSION AND CONCLUSIONS

Throughout this work, the anterior hypophysis of but one species of animal, the ox, was used in preparing the two types of thyrotropic extracts administered. Both types of extract contained other hypophyseal hormones. In each experiment, dosages of similar potency were given, as regards the thyrotropic hormone. Both stimulating and non-stimulating immunizing doses were

administered. One extract, the flavianate, rarely caused the development of a refractory condition of the thyroid, whereas the other, prepared by the commonly used sodium sulphate method, invariably induced a refractory state. But this refractoriness with the sodium sulphate preparation could be overcome by the flavianate preparation of the same hormone, from the same species, and of the same potency. The stimulation induced by the flavianate preparation was not due to the stimulating properties of the flavianic acid *per se,* as shown by control experiments.

The continued thyroid activation usually obtained with the hypophyseal flavianate, and the renewed stimulation of the thyroid of a refractory animal upon change of extract rule out several explanations which have been advanced to account for the development of the refractory condition, namely, exhaustion of the thyroid, depression of the animal's own pituitary gland by the thyroxin secreted in the stimulated thyroid and, lastly, some type of interaction between various endocrine glands such as the adrenal and pineal, induced by the administration of hypophyseal hormones (38).

The formation of a true antihormone as a response to an excess of hormone cannot explain the appearance of refractoriness to only one of the 2 extracts used above, for dosages of equal potency of both extracts were given. The formation and action of a physiological antihormone should not depend on the methods used in preparing the extracts, or on the species of animals supplying the pituitary glands used for extraction. But in the work here reported the method of preparing the extracts has been shown to influence profoundly the development of a refractory condition, and also that of an inhibitory factor in the injected animals' serum. The different responses therefore seem difficult to explain on any basis other than that the preparation is acting as an antigen resulting in a neutralizing antibody.

These results were obtained with a species known to be poor in antibody formation, suggesting that if another species had been used both extracts might have resulted in equal titers of inhibitory substances. In fact, Evans (39) has noted in hypophysectomized rats that growth stimulation declined and then ceased in 20 to 60 days when a flavianate extract was injected, but could be again induced with a crude alkaline extract, a result which he states is not readily explained by the antihormone hypothesis. Although the significance of the finding was not explained, Friedgood found that 2 guinea pigs, which had become refractory to one batch of extract, showed a rise in B.M.R. when injected with another batch of the same extract. Other work not in accordance with the antihormone theory has been published. Twombly (40) has reported that completely inactivated P.U. extract was as effective in producing refractoriness as was the active preparation. Smith (41) was able to secure continued growth up to 120 days with implants of rat hypophysis into hypophysectomized rats. This finding has been recently extended by Katzman et al (42), to include the thyrotropic and gonadotropic principles. Evans (33) has found similar results with crude alkaline extract of beef hypophysis. No refractoriness was noted during these experiments. Thompson (43) and Brandt and Goldhammer (44) could not find inhibitory substances in the serum,

when the extracts were made from the same species.

DuShane, Levine, Pfeiffer, and Witschi (45) have found that the parabiotic union of a castrate female rat with a hypophysectomized female results in unabated stimulation of the ovaries of the latter, for the duration of the union. A similar result has been reported by Martins (46) and McCahey et al (47), with the parabiosis of a castrate and a normal rat partner. Fluhmann (48) and others (23, 49) have also reported that the gonadotropic antihormones are species, as well as hormone, specific. They, too, believe that an immune reaction to the injection of a foreign protein better explains refractoriness than a true antihormone response. Gordon (59) finds the source of anti-substance in the reticulo-endothelial system, the site of antibody formation. The species specificity of the anti-substance has been put into question by the recent work of Parkes and Rowlands (50) and Thompson and Cushing (51, 52). Experiments with precipitin tests and complement fixation reactions in refractory animals are inconclusive, and have led to interpretations both for and against immune reaction (47, 53 to 58).

The above work, as well as that reported in the paper, indicates that refractoriness is the result of an antigen-antibody reaction. If this indication is correct, it removes the significance of the reaction in normal physiology. Whereas the evidence for the thyrotropic hormone is particularly clear, the situation as regards the gonadotropic hormones is more complex due perhaps to their number.

SUMMARY

A sodium sulphate preparation of beef anterior hypophysis containing thyrotropic hormone causes refractoriness to develop in 10 of 11 guinea pigs treated up to 39 days. A flavianate preparation made from the hypophysis of the same species, on the other hand, produces stimulation in 21 of 26 guinea pigs when treated for 39 or more days. Small non-stimulating doses of the sodium sulphate preparation are as effective as large stimulating doses in producing refractoriness, although small doses of the flavianate preparation did not produce refractoriness in 3 of 5 guinea pigs so treated. No inhibitory substance against the flavianate preparation was found in the serum of 3 rabbits and 2 sheep treated for prolonged periods. Nine of 11 guinea pigs refractory to the sodium sulphate preparation of beef anterior hypophysis responded with renewed thyroid gland stimulation to the administration of the flavianate preparation from beef anterior hypophysis. An extract has been obtained which usually produces prolonged thyroid gland stimulation without the development of refractoriness. Refractoriness is thought to be an immune response to proteins associated with the hormone in the extracts.

The author expresses his gratitude and acknowledges his indebtedness to Dr. P. E. Smith.

REFERENCES

1. Loeb, L., and R. B. Bassett: Proc. Soc. Exper. Biol. & Med. **26**:860. 1929.
2. Aron, M.: Compt. rend. Soc. de Biol. **103**:148. 1930.
3. Schockaert, J. A.: Am. J. Anat. **49**:379. 1932.
4. Loeb L., R. B. Bassett and H. Friedman: Proc. Soc. Exper. Biol. & Med. **28**:209. 1930.
5. Loeb, L., and H. Friedman: Proc. Soc. Exper. Biol. & Med. **29**:172. 1931.
6. Siebert, W. G., and R. S. Smith: Proc. Soc. Exper. Biol. and Med. **27**:622. 1930.

7. Collip, J. B., and E. M. Anderson: Lancet **1**:76. 1934.
8. Friedgood, H. B.: Bull. Johns Hopkins Hosp. **54**:48. 1934.
9. Oehme, C., H. Paal and H. O. Kleine: Arch. f. exper. Path. u. Pharmakol. **171**:54. 1933.
10. Schockaert, J. A., and G. L. Foster: J. Biol. Chem. **95**:89. 1932.
11. Bruner, R. C., and P. Starr: J.A.M.A. **106**:248. 1936.
12. Hertz, R., and F. L. Hisaw: Am. J. Physiol. **108**:1. 1934.
13. Zondek, B.: Die Hormone des Ovariums und des Hypophysenvorderlappens. Pp. 343. Springer, Berlin, 1931.
14. Leonard, S. L., and P. E. Smith: Anat. Rec. **58**:175. 1934.
15. McPhail, M. K.: J. Physiol. **80**:105. 1933.
16. Anderson, D., and J. B. Collip: Lancet **1**:784. 1934.
17. Selye, H., J. B. Collip and D. L. Thomson: Proc. Soc. Exper. Biol. & Med. **31**:487. 1934.
18. Selye, H., C. Bachman, J. B. Collip and D. L. Thomson: Proc. Soc. Exper. Biol. & Med. **31**:1113. 1934.
19. Selye, H., J. B. Collip and D. L. Thomson: Proc. Soc. Exper. Biol. & Med. **31**:566. 1934.
20. Collip, J. B.: J. Mt. Sinai Hosp. **1**:28. 1934.
21. D'Amour, F. E., C. Dumont and R. G. Gustavson: Proc. Soc. Exper. Biol. & Med. **32**:192. 1934.
22. Collip, J. B.: Ann. Int. Med. **9**:150. 1935.
23. Hisaw, F. L., R. Hertz and H. L. Fevold: Endocrinology **20**:40. 1936.
24. Harington, C. R.: Lancet **228**:1261. 1935.
25. Oehme, C.: Klin. Wchschr. **15**:512. 1936.
26. Loeb, L.: Science **80**:252. 1934.
27. Max, P., M. M. Schmeckebier and L. Loeb: Endocrinology **19**:329. 1935.
28. Hertz, S., and A. Kranes: Endocrinology **18**:415. 1934.
29. Loeser, A.: Arch. f. exp. Path. u. Pharmakol. **163**:530. 1931.
30. Closs, K., L. Loeb and E. M. MacKay: J. Biol. Chem. **96**:585. 1932.
31. Foster, G. L., A. B. Gutman and E. B. Gutman: Proc. Soc. Exper. Biol. & Med. **30**:1028. 1933.
32. Van Dyke, H. B., and Z. Wallen-Lawrence: J. Pharmacol. & Exper. Therap. **40**:413. 1930.
33. Evans, H. M., et al: The Growth and Gonad-Stimulating Hormones of the Anterior Hypophysis, Memoirs of the University of California, Vol. 2, 1933.
34. Schoedel, W.: Arch. f. exp. Path. u. Pharmakol. **173**:314. 1933.
35. Leipert, T.: Biochem. Ztschr. **270**:448. 1934.
36. Farrant, R.: Brit. M. J. **1**:470. 1914.
37. Rowlands, I. W., and A. S. Parkes; Biochem. J. **28**:1829. 1934.
38. Engel, P.: Klin. Wchschr. **14**:970. 1935.
39. Evans, H. M., R. I. Pencharz and M. E. Simpson: Endocrinology **19**:509. 1935.
40. Twombly, G. H.: Endocrinology **20**:311. 1936.
41. Smith, P. E.: Am. J. Anat. **45**:205. 1930.
42. Katzman, P. A., N. J. Wade and E. A. Doisy: Endocrinology **21**:1. 1937.
43. Thompson, K. W.: Proc. Soc. Exper. Biol. & Med. **35**:634. 1937.
44. Brandt, R., and H. Goldhammer: Ztschr. f. Immunitätsforsch. u. exper. Therap. **88**:79. 1935.
45. DuShane, G. P., W. T. Levine, C. A. Pfeiffer and E. Witschi: Proc. Soc. Exper. Biol. & Med. **33**:339. 1936.
46. Martins, T.; Compt. rend. Soc. de Biol. **119**:753. 1935.
47. McCahey, J. F., D. Soloway and L. P. Hansen: Pennsylvania M. J. **39**:228. 1936.
48. Fluhmann, C. F.: Proc. Soc. Exper. Biol. & Med. **32**:1595. 1935.
49. Meyer, R. K., and K. Gustus; Science **81**:208. 1935.
50. Parkes, A. S., and I. W. Rowlands: J. Physiol. **88**:305. 1936.
51. Thompson, K. W.: Proc. Soc. Exp. Biol. & Med. **35**:637. 1937.
52. Thompson, K. W. and H. Cushing: Proc. Roy. Soc., London, s. B. **121**:501. 1937.
53. Bachman, C.: Proc. Soc. Exper. Biol. & Med. **32**:851. 1935.
54. Gustus, E. L., R. K. Meyer and J. H. Dingle: Proc. Soc. Exper. Biol. & Med. **33**:257. 1935.
55. Ehrlich, H.: Wien. klin. Wchnschr. **47**:1323. 1934.
56. Sulman, F.: J. Exper. Med. **65**:1. 1937.
57. Kindermann, V., and F. Eichbaum: Ztschr f. Immunitätsforsch. u. exper. Therap. **89**:230. 1936.
58. Gegerson, H. J., A. R. Clark and R. Kurzrok: Proc. Soc. Exper. Biol. & Med. **35**:193. 1936.
59. Gordon, A. S., W. Kleinberg and H. A. Charipper: Proc. Soc. Exper. Biol. & Med. **36**:484. 1937.

SERUM PROTEINS BEFORE AND AFTER OPERATIONS FOR HYPERTHYROIDISM

R. B. BROWN AND P. M. MECRAY
From the Surgical Clinic of the Hospital, and the Harrison Department of Surgical Research, University of Pennsylvania
PHILADELPHIA, PENNSYLVANIA

A review of the literature on the serum proteins in hyperthyroidism makes it evident that the information concerning the subject is not only incomplete but confused. It was our inability to find convincing data which led to the present review of the literature and the investigation, the results of which we are reporting.

In much of the literature, the term total protein is used without clarification as to whether the plasma, serum or total blood protein is being reported. Rowe (1) found no change in the serum protein in two thyrotoxic patients, Hanson and McQuarrie (2) found no significant change in rabbits after thyroid feeding; Ling and Chang (3) and later, Láng (4), reported similar findings when dogs were used as the experimental animals.

On the other hand, many investigators have reported a fall in the protein values in the thyrotoxic patient (5 to 18). Deusch (6) believed that the decrease in the serum protein paralleled the severity of the disease and the weight loss. Improvement following surgical or roentgen therapy resulted in a rise in the serum proteins. Similar results have been reported following thyroid feeding to the experimental animal (19, 20). However Jiménez-Díaz (21) reported a decrease in the plasma proteins in partially thyroidectomized dogs, and an increase in the plasma proteins when the same dogs were administered thyroxin.

To add to the confusion, contradictory statements have been published on changes in the albumen-globulin ratio in hypo- and hyperthroidism (9, 19 to 24).

The many factors which may introduce errors in protein determinations have been pointed out by Howe (23) and the many conditions which may frequently co-exist in a patient, and which affect the serum proteins in one direction or another have been carefully reviewed by Van Slyke and Peters (26). These and many other factors may account in part for the variation in the reported results. The wide variety of the methods used increases the confusion already existing.

METHOD

Total serum proteins have been studied in 24 patients with hyperthyroidism. Twenty-three of these had a diffuse toxic goiter, and one a toxic nodular goiter. No patient has been included in this report in which there was any

question as to the diagnosis. In all patients, the initial basal metabolic rate was +30 or above. Serum protein determinations were made pre-operatively and, in most instances, before any iodine was administered. Similar studies were made at intervals varying from 3 to 12 months after operation. In only one of the 24 patients was there any clinical or basal metabolic evidence of persistance of the thyrotoxicosis at the time the postoperative determinations were made.

Pre-operative and postoperative blood samples were collected in an identical manner after an 8-hour period of total restriction of fluid and food intake. Ten cc. of whole venous blood were placed in a clean dry test tube and allowed to clot. Immediately thereafter, the serum was separated from the clot by centrifuging.

The determinations were made by the specific gravity method of Moore and Van Slyke (27). Serum was substituted for heparinized plasma. All determinations were made in duplicate. The limit of the error of the method is 0.6 gm. per 100 cc. of serum.

RESULTS

In table 1 are listed the pre- and postoperative serum protein values obtained from our group of 24 thyrotoxic patients. In 14 instances, the protein level increased after recovery from the thyrotoxicosis in excess of the error of the method. The rise was very slight in the other instances, but the mean increase of the entire group was 13 per cent. In 15 of the patients, the pre-operative serum protein value was below the normal accepted range, and in each instance the postoperative concentration was within the accepted normal range. The average pre-operative value for the group very closely approaches the low normal figure. All of the postoperative figures can be accepted as falling within the normal range.

We are well aware of the fact that many of the differences between the pre-operative and postoperative concentrations are within the maximum value for the error of the method, but it is the consistency of increase rather than its magnitude which we consider significant.

We have attempted a correlation of the serum protein values with several factors which might conceivably influence them. Although the results of this study have been interesting, the individual groups are perhaps too small to be of value in drawing definite conclusions. Furthermore, the inability to control the many factors affecting the serum protein concentration makes such a correlation without value unless the data are striking.

Howe (23) reported that he found lower plasma protein values in young rather than in old animals. The thyrotoxic patients we have studied fail to disclose such a relationship with age. Howe further stated that sex differences were not apparent. In our series, the pre-operative average for the 8 men was slightly but definitely lower (5.86 gm. per cent) than that for the 16 women (6.29 gm. per cent), but again these differences may not be significant.

Labbé and his associates (9), Bonilla and Moya (24) and Di Benedetto (17) failed to find any relationship between the serum protein concentrations

and the B.M.R. On the other hand, Loeper and his co-workers (10) reported that the fall in serum proteins paralleled the rise in the B.M.R. in treated hypothyroid patients. Deusch (6) also found in patients with Basedow's disease a parallel betwen serum protein values and the severity of the disease, especially the loss of weight. In our series these factors have been considered, and although there would appear to be an inverse relationship between the serum protein values, on the one hand, and the B.M.R. and percentage of the weight loss on the other, the differences are not large enough to be con-

TABLE 1. SERUM PROTEIN VALUES

Patient	Before Operation	After Operation	Difference	Difference
	gm./100 cc.	gm./100 cc.	gm./100 cc.	per cent
E.D.	6.78	7.20	+0.42	+ 6
L.G.	5.98	6.86	+0.88	+15
P.H.	5.75	7.30	+1.55	+27
C.M.	5.83	6.95	+1.12	+19
M.M.	5.98	6.60	+0.62	+10
E.H.	6.17	7.04	+0.87	+14
J.A.	6.04	6.88	+0.84	+14
P.B.	6.78	6.85	+0.07	+ 1
J.D.	6.20	7.92	+1.72	+28
R.P.	6.50	6.80	+0.30	+ 5
D.H.	6.96	6.86	−0.10	− 1
M.K.	6.55	6.87	+0.32	+ 5
E.S.	5.81	6.20	+0.39	+ 7
D.G.	6.45	6.99	+0.54	+ 8
L.Z.	5.79	8.20	+2.41	+42
I.G.	5.85	7.22	+1.37	+23
J.M.	6.13	7.34	+1.21	+20
G.K.	6.05	6.17	+0.12	+ 2
R.W.	6.75	7.20	+0.45	+ 7
G.W.	6.16	7.20	+1.04	+17
M.L.	5.72	6.86	+1.14	+20
L.L.	5.40	6.50	+1.10	+20
M.D.	5.63	6.86	+1.23	+22
S.B.	6.28	6.50	+0.22	+ 4
Mean	6.15	6.97	+0.82	+13

sidered significant. The mean serum protein concentration in the group with B.M.R. between +30 and +50 was 6.29 gm. per cent, whereas in the group with B.M.R. between +70 and +90 it was 6.06 gm. per cent. The mean serum protein concentration for the group which did not lose more than 10 per cent of the body weight was 6.24 gm. per cent, and for those losing more than 20 per cent it was 5.98 gm. per cent.

No relationship was found between the pre-operative serum protein levels and the duration of the symptoms of hyperthyroidism, and the same is true for the percentage increase of serum proteins after operation, with percentage weight gain. The mean increase in serum protein concentration was 13, 15 and 14 per cent in patients gaining up to 10, 20 and more than 20 per cent respectively, of body weight following operation.

DISCUSSION

The marked loss of weight which many of the hyperthyroid patients suffer, even though the appetite may be excessive, is an expression of their greatly increased metabolic activity. In patients with poor appetites tissue breakdown may be very considerable. These is little doubt in our minds that the lower serum protein concentrations found in our patients during the hyperthyroid state are an expression of the degree of malnutrition and protein starvation existing at that time. The data are thus in agreement with those of Bruckman, D'Esopo and Peters (28). Since many of the hyperthyroid patients lose considerable amounts of fluid through the skin, it is highly likely that the true serum protein values in the pre-operative period were even lower than those we obtained by maintaining the standard conditions for collecting the blood before and after operation.

While the serum protein concentration tends to become increased also by diarrhea and vomiting, conditions which may be present in severe hyperthyroidism, malnutrition may lead to a protein deficit. The interplay of the various factors influencing the proteins in one direction or the other undoubtedly accounts for the fact that the final concentration may vary considerably. It is quite likely that there is not only a reduction in the serum protein concenration during thyrotoxicosis, but a simultaneous reduction in the total serum protein coincident with a decreased blood volume which exists in many of these patients.

The rôle which the serum protein level may play in gastric secretion and gastro-intestinal motility we will discuss in a paper dealing primarily with the gastro-intestinal studies made on this same group of hyperthyroid patients.

SUMMARY

Serum protein determinations have been made both pre-operatively and postoperatively in a group of 24 hyperthyroid patients. In 14 of the group, the concentration of the serum proteins rose after operation in excess of the error of the method; in 15 of the 24 cases the pre-operative level was below the accepted normal concentration. An attempt was made to correlate the serum protein concentration with age, sex, B.M.R., weight loss, duration of symptoms, and weight gain following operation. Although suggestive trends have been found, the findings are not significant.

REFERENCES

1. Rowe, A. H.: Arch. Int. Med. **19**:354. 1917.
2. Hanson, S., and I. McQuarrie: J. Pharmacol. & Exper. Therap. **10**:261. 1918.
3. Ling, S. M., and H. Chang: Chinese J. Physiol. **4**:375. 1930.
4. Láng, S.: Ztschr. f. ges. exper. Med. **92**:102. 1933.
5. Leist, M.: Wien. Arch. f. inn. Med. **2**:491. 1921.
6. Deusch, G.: Deutsches Arch. f. klin. Med. **138**:175. 1921-22.
7. Frowein: Quoted by Deusch (6).
8. Curschmann, H.: München. Med. Wchnschr. **75**:425. 1928.
9. Labbé, M., F. Nepveux and I. Ornstein; Rev. franc. d'endocrinol. **10**:97. 1932.
10. Loeper, M., P. Soulié and J. Tonnet: Progrès méd. p. 198. 1934.
11. Loeper, M., J. Tonnet and Mlle. Lebert: Compt. rend. Soc. de biol. **101**:424. 1929.
12. Loeper, M., A. Lemaire, A. Lesure and J. Tonnet: *ibid.*, **101**:856. 1929.

13. Loeper, M., A. Lemaire and J. Tonnet: *ibid.*, **102**:279. 1929.
14. Loeper, M., A. Lemaire and J. Tonnet: *ibid.*, **104**:1200. 1930, *ibid.*, Progrès med. **2**:1965. 1930.
15. Loeper, M., A. Lemaire and J. Tonnet: Compt. rend. Soc. de biol. **104**:1201. 1930.
16. Loeper, M., A. Lemaire and J. Tonnet: Bull. et mém. Soc. méd. d. hôp. de Paris. **53**:1206. 1929.
17. Di Benedetto, E. J.: Compt. rend. Soc. de biol. **113**:452. 1933.
18. Shirer, J.: Am. J. M. Sc. **186**:73. 1933.
19. Rossignoli, J. J., Mlle. E. Di Benedetto, Mlle. I. Guerrero and E. J. Di Benedetto: Compt. rend. Soc. de biol. **113**:450. 1933.
20. Parhon, C. I., and L. Ballif, Compt. rend. Soc. de. biol. **95**:786. 1926.
21. Jiménez-Díaz, C.: Rev. méd. de Barcelona. **12**:26. 1929.
22. Starlinger, F.; Wien klin. Wchnschr. **37**:617. 1924.
23. Hoew, P. E.: Physiol. Rev. **5**:439. 1925.
24. Bonilla, E., and A. Moya: Rev. franc. d'endocrinol. **9**:93. 1931.
25. Maranon: Quoted by Bonilla and Moya (24).
26. Peters, J. P., and D. D. Van Slyke: Quantitative Clinical Chemistry, Vol. I, Interpretations, Williams & Wilkins, Baltimore, 1931.
27. Moore, N. S., and D. D. Van Slyke: J. Clin. Investigations, **8**:337. 1930.
28. Bruckman, F. S., L. M. D'Esopo and J. P. Peters: J. Clin. Investigation **8**:577. 1930.

THE SEX HORMONE EXCRETION OF CHILDREN

RALPH B. OESTING[1] AND BRUCE WEBSTER
From the Barbara Henry Research Laboratory of the New York Hospital, and the Department of Medicine, Cornell University Medical College
NEW YORK CITY

Koch (1) in summarizing the findings of his research group stated that there are marked daily fluctuations in the urinary excretion of androgens and estrogens in normal men and women. He found an average daily excretion of 60 international units of androsterone in men and 50 I.U. in women. He gave no data on children. He found as an average daily excretion of estrogens, expressed as estrone, 9 to 12 γ for men and 18 to 36 γ for women. In male castrates he found exceedingly small amounts of sex hormones in the urine.

Kochakian (2) has not expressed his units in terms of any standard; however, he found that young men excreted 6 to 36 capon units daily with an average of 14 C.U. Old men excreted less hormone. He was unable to recover more than 6 per cent of the androstendion injected into either men, rabbits, or dogs. Pedersen-Bjergaard (3) did not define the size of his capon unit but showed a hormone excretion for a normal adult male of 15 to 25 C.U. and gave the excretion of an 8-year-old boy as less than 3 C.U.

Dingemanse, Borchardt and Laqueur (4) have studied with adequate methods the hormone excretion of males and females. They expressed their results in international units of androsterone. For men from 20 to 40 years of age they found an average daily androgen excretion of 45 I.U. with a range from 15 to 170 I.U. For women of active sexual life they found a daily excretion of 30 to 60 androgen units. In old men they found 30 U or more being excreted daily while in boys of ages 5 to 15 years they found up to 15 U being excreted. No cycle was found in either men or women in the rate of androgen excretion in a study extending over a period of 45 days. Many valuable data were given on methods, for details of which the reader is referred to the original report.

Dorfman and Greulich (5) in an advance abstract gave the following: "The urines of 18 boys and 5 girls, varying in ages from 6 to 16 years, were assayed for androgen and estrogen activity. The androgenic activity varied from 1.1 to 32 I.U. per 24 hours, while the estrogenic activity ranged from less than 5 to 95 I.U. of estrone for the same period. Attention is called to the difference in sex hormone excretion of children who, though of the same chronological age differ from each other in the degree of their physical maturity."

This survey of the recent literature on hormone excretion in man prepares us to examine critically the data of the present report.

[1] General Education Board Fellow.

MATERIALS AND METHODS

The children used in this study were cases from the childrens' pavilion of the New York Hospital together with children from an orphanage. They were normal, or were considered by us to have recovered from a non-febrile ailment which did not involve the endocrine system of the child. Twenty-four-hour urine specimens were collected from each child and subjected to analysis for androgenic and estrogenic activity, according to the methods described below. No preservative was added to the urine.

If the 24-hour collection represented less than 1500 cc. it was diluted to that volume, and if more than 1500 cc. an aliquot of the total sample was taken. However, the results were always calculated to a 24-hour basis. To the 1500 cc. of urine was added with stirring 50 cc. of concentrated H_2SO_4. The acidified urine was heated in an autoclave for 10 minutes at 15 pounds steam pressure. After cooling rapidly the urine was exhaustively extracted with benzene in a continuous extractor. The benzene was distilled off under vacuum and the residue taken up in ether and transferred to a separatory funnel where the ether solution was shaken 5 times with 10 per cent aqueous solution of NaOH (10 gm. per 100 cc.) using 25 cc. each time. This treatment was followed by washing 3 times with 30 cc. portions of distilled water and combining the water washings with the NaOH solution. This procedure was found adequate to separate the androgenic and the estrogenic activity of the urine.

The residual ether solution, containing the androgenic activity, was stirred for a few minutes with 0.5 gm. of Norit-A (decolorizing charcoal) and filtered into a distilling flask. The charcoal was washed with fresh ether and this also was filtered into the flask. The ether was evaporated off and the residue dissolved in a measured volume of 60 per cent ethyl alcohol (60 cc. of 95 per cent alcohol diluted to 95 cc.). The androgenic activity of the urine specimen was determined on this solution, according to the methods described by one of the authors (6). Further data on this method will be presented later in this report.

Thirty-five cc. of concentrated HCl was added to the NaOH extract and washings which contained the estrogenic activity. After cooling, this acidified solution was extracted 5 times with 100 cc. portions of ethyl ether and the ether extract washed with 100 cc. of saturated sodium bicarbonate followed by distilled water. The ether was evaporated off and the residue taken up in a measured volume of olive oil. The oil solution containing the estrogenic activity of the sample was titrated on ovariectomized mice. A mouse unit was found to be equivalent to 2 I.U. of estrone.

When titrating an unknown urine extract which is probably a mixture of estrogens, it is difficult to evaluate the significance of the results for estrogenic activity expressed as international units of estrone. The method of titration may aid in interpreting the results and is therefore given. One must also consider the species of animal used for the titration. Oil solutions of the standard were injected subcutaneously into the backs of white mice. Two weeks after the removal of both ovaries each mouse was given the equivalent of 10 I.U. of estrone in a single injection and only those mice which showed completely cornified vaginal smears within 72 hours after the injection were used for subsequent work. These mice were each given the equivalent of 2 I.U. units of estrone in one injection at weekly intervals for the next 3 weeks, and again only those mice which showed completely cornified smears within 72 hours after each injection were used for a titration. One week

after the third injection of the standard, the mice were divided into groups of 3 and given varying doses of the urine extract being titrated. A completely cornified smear on each of the mice in a group was considered a positive response. It was not possible to determine accurately by this method less than 10 I.U. of estrone as the 24-hour excretion of a child. It was often apparent by the partial reaction of a group of 3 mice that some estrogenic activity was present. In these cases estrogenic activity was recorded, but as less than 10 I.U. per 24 hours.

The lower limitation of 10 I.U. per 24 hours was imposed on us because the total activity of an extract cannot be taken up in less than 2 cc. of oil. For the same reason a plus or minus value of 4 I.U. must be assigned to each 24-hour titration.

TABLE 1. THE CORRELATION BETWEEN COMB GROWTH OF CAPONS AND COLOR UNITS

Color Units	Increase in Comb Area[1]	Color Units	Increase in Comb Area[1]	Color Units	Increase in Comb Area[1]
trace	4.5	4.0	38.0	6.2	41.0
.24	5.0	4.0	48.0	7.2	46.0
.75	18.0	4.2	35.8	7.2	52.0
1.0	20.0	4.6	44.0	7.2	78.0
1.1	28.0	5.2	38.0	7.2	80.0
1.4	18.4	5.2	58.0	7.8	53.0
2.0	24.0	5.4	48.0	8.1	81.0
2.0	29.0	5.6	56.0	8.4	46.4
2.4	38.0	5.8	40.0	10.8	80.0
2.8	26.0	5.8	42.0	10.8	86.0
3.2	34.0	6.0	53.6	10.8	95.0
3.2	38.0	6.1	72.0	11.4	60.0
				13.5	98.0

[1] Increase in comb area is expressed as the average increase in area of the combs of at least 2 capons for each result recorded. A color unit represents 10 ± 2 γ of androsterone.

The back of each mouse was massaged for a few minutes on the day following an injection to insure absorption of the hormone, and at no time was more than 0.2 cc. of oil given in a single injection. After the titration, the mice were again put on the standard for 2 weeks before being used for a second titration. After the second titration the animals were discarded. Many of the titrations on younger children by this method gave negative results.

In a preliminary report on the colorimetric assay for male sex hormones in urine (6), one of the authors had available only a few preliminary results, and in presenting these made rough comparisons between the two columns of data given. One column gave the growth of the capon's combs as recorded for an assay, while the other gave the number of color units per 24-hour specimen of urine and not the number of color units applied directly to the comb. Recalculation of these data together with a complete study of the method is given here.

Greenwood et al (7) have made a thorough study of the variables in the capon comb growth method of measuring androgenic activity, and for details the reader is referred to their paper. The data have been analyzed statistically and merit careful consideration. It was shown that the age of the capon had no effect on the result as long as the bird was a complete castrate. However, partial castrates gave a greater response than did complete ones. The daily response was not uniform, and a definite duration of the assay was essential. They gave data on the relation of dose to response when intra-

muscular injection was used. They showed that there was no priming effect such as exists for estrone assays, and that the nature and amount of substrate injected did affect the results.

Fussgänger (8) described an inunction assay for androgen activity using capons. The hormone was painted directly on the comb as an oil, alcohol or water solution. The author was able to produce a 30 per cent increase in comb area with 1/50 of the dose used to produce this same response when injected intramuscularly. We found that the application of an alcohol solution of the hormone to the comb was most satisfactory, as this solution would quickly dry into the comb without loss or ill effects upon it.

Dessau (9, 10) and Dessau and Freud (11) have further studied the

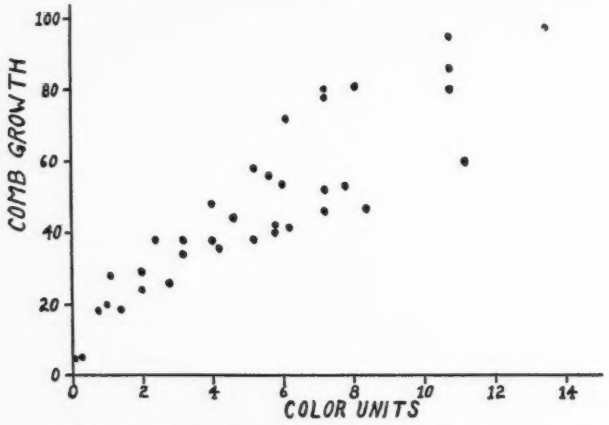

Fig. 1. Correlation between comb growth and color units.

inunction assay with confirmatory results as to the sensitivity. One result of their work is of particular interest to us. It was shown that the growth resulting from the application of androsterone, testosterone and of androstendion directly to the comb was of similar order, and not so widely different as was found when these androgens were injected intramuscularly. This is an important point in interpreting the results of an assay. The histological development of the comb was found to be the same when treated by inunction as when intramuscular injection was used.

We have developed the Fussgänger capon assay along the following lines as a working tool for our colorimetric assay. The growth of the comb was determined by measuring with a planimeter the area of a shadow photograph of the comb taken under standard conditions. Our standard deviation, calculated from 5 measurements each on a group of 10 capons, according to the statistical methods of Fisher (12) was ± 3.9 per cent. Interpreting this figure into Fisher's tables of t, a comb unit of significant size when only 2 birds are used for a point in an assay must be a minimum average increase in comb area of 15 per cent. With a comb unit thus determined we found over a series of assays that this unit corresponded to 10 γ of androsterone

given in 5 daily doses of 2 γ onto each capon's comb. All applications were made with 60 per cent alcohol which quickly dried into the combs. A tuberculin syringe with a 27 gauge needle was used to apply the hormone solution to the comb surface.

Zimmerman (13, 14) described a colorimetric reaction for the sex hormones, and gave a method for determining the hormone content of urine

TABLE 2. THE CORRELATION BETWEEN URINE VOLUME PER 24-HOUR SPECIMEN AND THE NUMBER OF COLOR UNITS CONTAINED IN THE SPECIMEN

Color Units	Urine Volume cc.	Color Units	Urine Volume cc.	Color Units	Urine Volume cc.
trace	590	56.0	1120	136.0	800
3.2	820	56.0	1390	144.0	490
11.2	450	64.0	610	152.0	640
24.0	420	80.0	700	160.0	550
24.0	950	93.0	400	160.0	620
32.0	460	96.0	730	160.0	680
32.0	1160	112.0	600	160.0	1060
40.0	410	112.0	1000	173.0	1800
40.0	800	120.0	1320	180.0	1050
40.0	1210	128.0	560	187.0	1560
48.0	1170	128.0	900	240.0	900
56.0	600	128.0	950	240.0	1435
				300.0	1100

using the Pulfrich photometer for color measurements. However, he did not give biological assays on the urine extracts to confirm the chemical assays. These data are essential, due to the non-specific reaction involved. In applying the method to our research problem (6), we developed a colorimeter (now

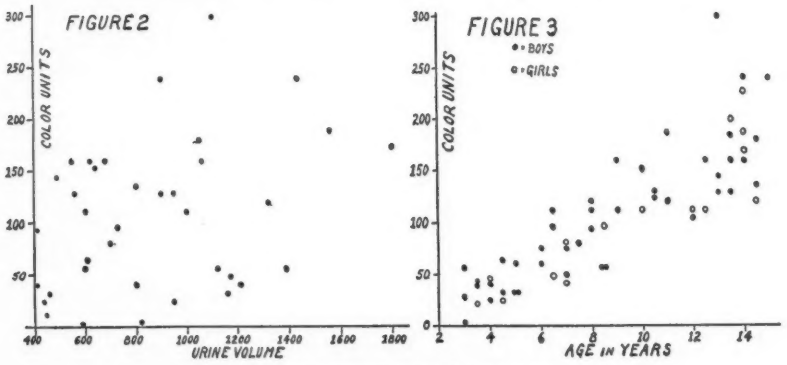

Fig. 2. Correlation between color units and urine volume.
Fig. 3. Correlation of age and androgen excretion.

manufactured by Hellige, Inc. of New York) which was simpler than the photometer and, we believe, equally effective. Other changes in our procedure are described in the preliminary report (6). The colorimeter was designed and calibrated by a series of biological assays on capons to measure a color unit of the same size as our capon unit first described. This development

was essential, as both units must lie in the same range in order to make significant comparisons. This is a point we wish to stress, as the development of the colorimeteric method was possible only after the development of the more accurate biological assay. One color unit is equivalent to 0.1 I.U. of androsterone.

To test the colorimetric method for losses, a benzene extract was divided into 2 equal aliquots. One was evaporated down and made up immediately for assay in 60 per cent alcohol. The other was run through the procedure described for clearing the sample for color work and then assayed at the same time as the first aliquot. Each showed the same androgenic activity by

TABLE 3. THE HORMONE EXCRETION OF BOYS BETWEEN THE AGES OF 3 AND 15 YEARS

Age, yr.	Male hormone in color units per 24 hr.	Female hormone, as I.U. of estrone per 24 hr.	Age, yr.	Male hormone in color units per 24 hr.	Female hormone, as I.U. of estrone per 24 hr.
3	trace	—	8.5	56	—
3	27	—	8.5	56	—
3	56	—	9	112	—
3.5	40	—	9	160	—
3.5	40	—	10	152	—
4	24	—	10.5	124	—
4	40	—	10.5	128	—
4.5	32	—	11	120	—
4.5	64	—	11	187	—
5	32	—	12.5	160	<10
5	32	—	13	128	<10
5	60	—	13	144	—
6	60	—	13	300	30
6	75	—	13.5	128	<10
6.5	96	—	13.5	160	60
6.5	112	—	13.5	173	10
7	48	—	14	160	20
7	75	—	14	240	12
7.5	80	—	14.5	136	45
8	93	—	14.5	180	<10
8	120	—	15	240	50

biological assay. This experiment was repeated a second time to be sure there was no appreciable loss in activity by the treatments involved. It was thought that too much charcoal used in clearing the samples might result in a loss of androgens. We have not found this to be the case when a 0.5 gm. was used.

In table 1 and figure 1 are given the results of an extensive series of assays on urine extracts. These data are given in detail to show the range that is encountered when the variation of the biological assay and the variation from impurities carrying over in the colorimetric assay are combined in practical work. In a nonspecific method of which we have many in biochemical work, this test is the final proof of the practical value of the method. Each point on the figure represents a 24-hour specimen of urine from a separate individual on which a biological as well as a chemical assay was run. All of these individuals were children used in work reported in the main body of this paper.

RESULTS

In the course of our studies on methods, we became interested in the possibility of a correlation between the volume of a 24-hour specimen and the number of color units found in it. The data of table 2 as plotted in figure 2 show that there is no correlation between these two variables. This is an interesting finding when we remember that a variation in the daily excretion of androgens by individuals (1) has been demonstrated. We decided that an expression of hormone excretion on a 24-hour basis and not on a volume basis would be more valuable in our study of children. This we believe is the more accepted approach.

We have collected 24-hour urine specimens from 43 boys and 16 girls between the ages of 3 and 15 years, and examined these for their androgenic

TABLE 4. THE HORMONE EXCRETION OF GIRLS BETWEEN THE AGES OF 3 AND 15 YEARS

Age, yr.	Male hormone in color units per 24 hr.	Female hormone as I.U. of estrone per 24 hr.	Age, yr.	Male hormone in color units per 24 hr.	Female hormone as I.U. of estrone per 24 hr.
3.5	20	—	12	104	<10
4	44	—	12	112	20
4.5	24	—	12.5	112	50
6.5	48	—	13.5	200	80
7	40	—	14	168	50
7	80	—	14	187	80
8.5	96	—	14	227	30
10	112	<10	14	120	30

and in some cases for their estrogenic content. In the younger children no assay for estrogens is reported, as it was found that our present method was not sensitive enough to detect the amounts excreted at these ages.

Table 3 summarizes these data on the boys. The age is given in years to the nearest half-year, and the androgenic hormone content is expressed in color units. To convert these to international units divide the value given in color units by 10. The estrogenic hormone content is expressed as international units of estrone.

Table 4 contains the data for the girls expressed in the same units as table 3. The combined data for androgen excretion are presented as a graph in figure 3.

Examination of these data shows that there is a gradual rise in male hormone excretion from early childhood on through puberty, since the average adult produces 40 to 50 I.U. units of androgen per 24 hours (1, 4). The much smaller amounts of estrogen produced with the distinct rise at puberty lead one to the hypothesis that puberty as effected by androgens is a somatic threshold reaction, and that the increase in estrogens at puberty and their action combined with male hormone plays a more important part in the development of the adult form and in the closing of the epiphyses and other changes of puberty. This point is emphasized by the observation, in table 3, that boys do not excrete estrogens in measurable amounts until they

reach the age of 13 or more, while in table 4 we note that girls of 10 to 12 are excreting estrogens. This observation might indicate the earlier arrival of puberty in girls, but confirmation would be required before this difference is accepted.

In rats it has been shown that the injection of ovarian hormones inhibit somatic growth, while large amounts of male hormone (testosterone) when injected into young animals had no such inhibiting effect (15). Further evidence on this point is brought to our attention by the results of Seeman (16). Using the immature guinea pig, he found a more rapid closing of the epiphyses in castrated males and females when injected with estrogen-containing extracts of boar's urine. Furthermore, Hooker (17) has shown that in bulls there is no sharp pubertal rise in androgen urinary-excretion, but that there is a gradual rise from the age of one month on.

Present knowledge of hormone excretion is by no means adequate to an understanding of the endocrine changes of puberty.

SUMMARY

Complete data are given on a colorimetric assay for male hormones in urine.

The androgen and estrogen excretion of 43 boys and 16 girls has been studied. There is a gradual rise in androgen excretion from early childhood to adult life. At puberty there is a distinct rise in estrogen excretion. We did not find this to be the case for androgens. It is suggested that estrogens play a more significant rôle in epiphyseal closure than androgens.

REFERENCES

1. Koch, F. C.: Ann. Int. Med. **11**:297. 1937.
2. Kochakian, C. D.: Endocrinology **21**:60. 1937.
3. Pedersen-Bjergaard, K.: Zentralbl. f. Gynäk. **60**:372. 1936.
4. Dingemanse, E., H. Borchardt and E. Laqueur.: Biochem. J. **31**:500. 1937.
5. Dorfman, R. I., and W. W. Greulich: Endocrinology **21**:741. 1937.
6. Oesting, R. B.: Proc. Soc. Exper. Biol. & Med. **36**:524. 1937.
7. Greenwood, A. W., J. S. S. Blyth and R. K. Callow: Biochem. J. **29**:1400. 1935.
8. Fussgänger, R.: Medicine in its Chemical Aspects **2**:185. 1934.
9. Dessau, F.: Acta. brev. Neerland **5**:94. 1935.
10. Dessau, F.; Acta. brev. Neerland **5**:139. 1935.
11. Dessau, F., and J. Freud: Acta. brev. Neerland **6**:9. 1936.
12. Fisher, R. A.: Statistical Methods for Research Workers, 3rd ed., Oliver & Boyd, Edinburgh. 1930.
13. Zimmermann, W.: Ztschr. f. physiol. Chem. **233**:257. 1935.
14. Zimmermann, W. Ztschr. f. Physiol. Chem. **245**:47. 1937.
15. McEuen, C. S., H. Selye and J. B. Collip: Proc. Soc. Exper. Biol. & Med. **36**:390. 1937.
16. Seemann, H. Endokrinologie **18**:225. 1937.
17. Hooker, C. W.: Endocrinology **21**:655. 1937.
18. Wu, H., and C. Y. Chou: Chinese J. Physiol. **11**:413. 1937.
19. Wu, H., and C. Y. Chou: Chinese J. Physiol. **11**:429. 1937.
20. Callow, R. K., N. H. Callow and C. W. Emmens: J. Soc. Chem. Industry **56**:1056. 1937.

IS THE BLOOD CALCIUM LEVEL OF MAMMALS INFLUENCED BY ESTROGENIC SUBSTANCES?[1]

LOUIS LEVIN[2] AND PHILIP E. SMITH
From the Department of Anatomy, College of Physicians and Surgeons, Columbia University
NEW YORK CITY

The literature on the relationship of the gonads to the level of calcium in the blood has recently been reviewed by Zwarenstein (1) and by Marlow and Koch (2). A study of the results which have been reported leads to the impression that the gonads (i.e. the gonadal hormones) have very little, if any, influence on the level of the serum calcium in mammals.

In a recent communication, Riddle and Dotti (3), on the basis of results obtained by administering various estrogens to pigeons, doves, rats, and dogs, conclude that these substances, particularly theelin and dihydrotheelin benzoate, are potent agents in raising the serum calcium level. The actual increases in calcium level found by these investigators were small, except in the experiments on pigeons and doves. Furthermore, the data reported were obtained from very small groups of animals.

On the basis of these results, Riddle and Dotti have suggested several implications relating the estrogenic substances, via the calcium balance, to sexual and racial differences in stature and to the "benefit currently derived from theelin administration in some mental cases." Also, the existence of a parathyrotropic hormone of the pituitary is questioned.

The work of Riddle and Dotti was the first[3] to indicate that the pure estrogenic substances cause an increase in serum calcium, and since such far-reaching and important implications were made on the basis of this work it seemed desirable that a confirmation of the findings be attempted. We have consequently studied the blood calcium in rats, rabbits and monkeys before and after the administration of estrogenic material. The findings are presented in this communication.

METHODS

The animals employed were placed on a constant diet, other conditions being maintained as constant as possible. The estrogenic material was injected subcutaneously as a sesame oil solution of either theelin or dihydrotheelin,[4] specified in appropriate places. These two compounds, according to Riddle and

[1] Aided by grants from the Committee for Research in Problems of Sex, the Nationl Research Council and from the Rockefeller Foundation, New York City.
[2] E. R. Squibb and Sons Fellow.
[3] Saviano (4) is cited by Riddle and Dotti as having found increased serum calcium in 2 dogs as a result of treatment with progynon.
[4] The theelin and the estradiol benzoate (progynon-B) were furnished by the Schering Corporation, through the courtesy of Dr. Erwin Schwenk.

Dotti, are the most potent of the estrogenic substances in ability to raise the blood calcium level.

Control calcium values were obtained either before treatment with estrogen was begun, or from the blood of untreated animals taken at the same time as the experimental bloods. In one series of rabbits, and in the monkey series, both types of control values were obtained. Blood was always obtained by cardiac puncture without anesthesia, except in the case of rats which were lightly anesthetized with ether when the blood was taken.

TABLE 1. EFFECT OF THEELIN ON RAT SERUM CALCIUM

Litter Number	Body Wt. at Spaying	Age at		Serum Calcium			
		Spaying	First Injection	Non-Injected		Injected[1] 6 days	
				Normal	Spayed	Normal	Spayed
	gm.	days	days	mg. %	mg. %	mg. %	mg. %
I	143–156	69	82	—	12.3[2]	—	11.9[2]
IA	125–149	—	82	12.5[2]	—	13.0[2]	—
II	171–206	157	170	11.3	12.5	12.2	12.0
VII	175–191	141	161	12.6	12.1	13.3	12.8
IX	184–191	169	189	12.1	12.2	13.0	13.7
X	178–208	139	159	12.9	11.9	11.9	12.3
Mean ± mean dev. of mean				12.3±0.3	12.2±0.1	12.7±0.3	12.5±0.3
				Non-Injected		Injected[1] 12 days	
III	174–208	161	181	11.7	12.5	12.8	12.2
IV	188–196	147	167	12.8	12.7	12.8	14.3
V	170–199	147	169	12.8	11.8	13.4	14.0
VI	190–230	167	189	12.5	12.4	11.9	12.4
XII	180–210	166	186	11.6	14.1	12.8	13.5
Mean ± mean dev. of mean				12.3±0.3	12.7±0.4	12.7±0.2	13.3±0.4
Grand Average				12.3	12.5	12.7	12.9

[1] Injected animals received 100 γ daily.
[2] Values obtained from pooled blood of 2 rats, the animals being too small to furnish individually sufficient serum for analysis.

Serum calcium was determined by the Clark and Collip (5) modification of the Kramer and Tisdale method. Duplicate determinations were made on each serum. The reagents were checked each time they were used.

RESULTS

Forty-four female rats, 11 litters of 4 rats each, were placed on a mixed diet and kept under standard conditions for several days, and then 2 rats of each litter were bilaterally ovariectomized. Treatment with estrone in oil was begun 13 to 22 days later, one castrate and one intact rat of each litter receiving 100 γ of theelin daily. On the seventh day, after each of the injected rats had received a total of 600 γ of theelin, blood was taken from half the litters, and the serum calcium of each rat determined.

The remaining litters were continued as previously until each of the injected rats had received 12 daily injections of 100 γ of theelin. On the 13th

day, blood was taken from these animals and their serum calciums determined. The results of these determinations are given in table 1.

As is obvious from an inspection of these data, the mean serum calciums of the 8 groups of rats are nearly identical. In only one case is an appreciable difference to be noted, namely, between the unoperated controls and the ovariectomized 12-day injected animals. Even this difference, 1.0 mg. per cent, would not usually be considered significant. Statistically, the value of 't'[5] for this difference is 2.012, indicating a probability of approximately 8 in 100 that the difference is due to random sampling. It is therefore apparent that in

TABLE 2. EFFECT OF ESTRADIOL BENZOATE ON SERUM CALCIUM OF OVARIECTOMIZED RABBITS

	Control (Non-Injected)		Injected 1000 I.U. daily	
	Sample 1 3/8/37	Sample 2 3/15/37	15 days 4/13/37	31 days 4/29/37
	mg. %	mg. %	mg. %	mg. %
Rabbit 1	13.95	14.55	13.50	14.45
2	12.40	14.75	14.75	15.30
4	13.40	14.00	14.90	13.70
5	14.45	13.55	15.20	14.55
6	14.50	14.75	14.70	14.90
7	13.45	13.65	14.75	14.40
8	13.95	14.80	15.05	14.90
9	13.15	12.80	14.65	14.40
10	14.45	13.65	15.75	14.55
Mean $\pm \epsilon_M$[1]	13.74±0.24	14.06±0.23	14.81±0.20	14.57±0.15
	13.90±0.17		14.69±0.12	

[1] ϵ_M = Mean deviation of the mean.

these experiments the serum calcium level was not influenced by the injection of theelin in relatively large doses.

Since a great deal of work has been done on the effect of the ovary on the serum calcium of rabbits, and since the results of such work are in considerable disagreement, we decided to determine the effect, if any, of a crystalline estrogenic compound, estradiol, on the serum calcium of this species.

The first experiment was carried out on a group of 9 adult ovariectomized female rabbits. The ovariectomies had been performed several months before, and the animals had been kept in the same cages and under the same standard conditions for an even longer period. The control serum calcium level was determined twice on each animal, one week intervening between the two determinations. Thirteen days after the last control blood was taken, injections of estradiol benzoate were begun, each animal receiving 1000 I.U. daily. The injections were continued for 31 days, the blood calcium being determined after 15 injections and after the complete course of 31 injections. The data obtained are given in table 2.

[5] The probability that random sampling is responsible for a difference between 2 means is usually estimated from the ratio of the difference to its probable error. For small numbers of observations a more accurate estimate of the probability (P) is obtained by calculation of the value designated as 't' by Fisher (6). A probability of 0.01 indicates one chance in 100 that random sampling is responsible for the difference between the two means.

If one scrutinizes the data, it may be noted that the changes in the calcium values for the individual animals showed neither qualitative nor quantitative constancy. Some rabbits (No. *5, 9, 10*) show what appear to be increases in serum calcium after 15 days of treatment. On the other hand, rabbit *1* shows a fall in calcium of similar degree. Still others (No. *2, 6, 8*) show practically no change. Similarly, when injections were continued for 16 more days, the calcium levels of some animals showed further increase, some a decrease, and others remained constant. It should be emphasized, however, that in all cases where apparent change occurred, it was relatively small.

If the mean values are compared, it may be seen that the greatest difference (between the mean of the first set of control determinations and that of the 15-day injected group) is 1.07 mg. per cent, a difference which, in the light of the individual variations shown, would ordinarily not be considered of great significance. If these means be compared statistically, it is found that the differences between the first mean control value and the mean values after 15 and 31 days of treatment are possibly significant ($P = <0.01$ and 0.01 respectively), while the differences between the mean of the second series of control values and those after the injections are probably not significant ($P = 0.03$ and 0.10 respectively). If the two control series are combined and also the two injected series, the mean values for the serum calcium are 13.90 and 14.69 mg. per cent respectively, the difference being only 0.79 mg. per cent. However, the value of '*t*' for this difference is 3.823, establishing *P* as less than 0.01, and therefore indicating the difference between the means to be statistically significant.

Since the estradiol seemed to have a possible elevating effect on the serum calcium of ovariectomized rabbits, another experiment was made employing non-castrated adult female rabbits. The conditions of the experiment were similar to those described above. As in the previous experiment, calcium determinations were made simultaneously on all the animals, 3 control determinations being performed on each rabbit at 1-week intervals. Half of the group was then given daily injections of estradiol benzoate, 1000 I.U. per rabbit per day, the remaining animals being continued as uninjected controls. Again, the calcium values were determined after 15 and 29 daily injections.

As indicated by the data (table 3) the serum calcium level in both groups of animals gradually decreased from week to week. During the first 2-week period, between the first and third control determinations, the mean values of the control group fell from 14.98 to 13.01 mg. per cent, a mean drop of 1.97 mg. per cent, while during the same interval the mean calcium level of the experimental group fell only 0.93 mg. per cent (from 14.49 to 13.56 mg. per cent). This indicates that whatever influence was causing the decrease in blood calcium was acting more powerfully in the control group than in the experimentals. The fact that during the next 2-week period (when the experimentals were being injected) the mean calcium value of the controls continued to decrease slightly (from 13.0 to 12.6 mg. per cent), while that of the experimentals increased slightly (from 13.56 to 14.01 mg. per cent) exaggerates the difference between the means for the 2 groups at

this time. This may partially explain why the value for 't' for the difference between these two means is as high as 6.98, establishing an extremely low value for P, and thus indicating that the difference is probably significant.

The difference between the means of the experimental group before and after the 15-day injection period is not significant ($t = 2.18$; $P = 0.05$), nor is that between the control group and the 29-day injected group ($t = 0.078$; $P = 0.90$). It appears, therefore, that if the estrin did have an elevating effect on the serum calcium of these animals, such elevation was only transitory, disappearing between the 15th and 29th day of treatment.

TABLE 3. EFFECT OF ESTRADIOL BENZOATE ON SERUM CALCIUM OF INTACT FEMALE RABBITS

	Serum Calcium values in mg. per cent				
	6/16/37	6/24/37	7/1/37[1]	7/21/37[1]	8/4/37[1]
Non-injected controls					
Rabbit 14	15.25	13.90	12.00	12.15	12.40
15	14.65	13.40	14.10	13.00	13.50
16	15.30	12.70	12.70	12.40	13.55
17	15.15	14.90	13.50	12.95	13.80
18	14.55	13.70	12.75	12.50	13.55
Mean $\pm \epsilon_M$	14.98±0.16	13.72±0.36	13.01±0.36	12.60±0.16	13.36±0.25
1000 I.U. daily for	0 days	0 days	0 days	15 days	31 days
Rabbit 11	14.70	13.20	13.20	14.25	12.25
12	15.10	14.50	13.95	14.20	13.65
13	14.05	13.70	13.20	13.90	13.95
19	13.35	13.50	13.50	13.60	13.60
20	15.25	14.80	13.95	14.10	13.20
Mean $\pm \epsilon_M$	14.49±0.35	13.94±0.30	13.56±0.17	14.01±0.12	13.33±0.30

[1] Fasted 20-24 hours before blood was taken for determination.

A further investigation of the effect of estrin on the serum calcium was made, using 6 young macacus monkeys as experimental animals, one control determination being performed on each animal. Four of the monkeys were then given daily intramuscular injections of estradiol benzoate, 2 receiving 10,000 I.U. and 2 receiving 5,000 I.U. daily. The other 2 animals were retained as untreated controls. Serum calcium was determined after 17 and 31 injections. Table 4 shows the data obtained.

It is apparent that the estrin treatment caused no elevation in the serum calcium level. In fact, after treatment was begun the calcium level decreased, but since the decrease was of the same order in the uninjected controls it appears to be due to some factor other than the estrin. The fact that these experiments were conducted during the hot summer months, which in the New York area are decidedly unfavorable for monkeys, may account for the fluctuations in the values observed.

That the monkeys did respond to the estrin with the usual symptoms was indicated by the very marked swelling of the external genitalia and the

mammary nipples shortly after institution of treatment. Also, at autopsy after the final calcium determination, when compared with those of the uninjected controls, the uteri of the injected animals were found to be much increased in size and weight.

DISCUSSION

The data presented in this communication show that the serum calcium of rats, rabbits and monkeys is only very slightly, if at all, affected by estrin administration or ovariectomy. The results obtained in rats, in particular, are difficult to harmonize with those of Riddle and Dotti. Administering the same substance (theelin), these investigators claim to have caused increases of 3.1, 1.5 and 2.5 mg. per cent, respectively, in the serum calcium of castrate, hypophysectomized castrate, and thyroidectomized castrate rats, as compared with the uninjected controls. What animals furnished the control

TABLE 4. EFFECT OF ESTRADIOL BENZOATE ON SERUM CALCIUM OF IMMATURE FEMALE MONKEYS

Serum calcium values expressed as mg. per cent

Days injected Date of determination		0 7/23/37	17 8/9/37	31 8/23/37
Uninjected controls	My 3	10.1	8.4	9.3
	My 4	10.4	9.5	9.4
5000 I.U. daily	My 5	9.1	7.9	[1]
	My 6	9.0	7.5	7.3[2]
10,000 I.U. daily	My 1	8.9	8.0	8.4
	My 2	9.6	7.2	9.4

[1] Monkey 5 was found dead after receiving 26 injections.
[2] Determination made after 26 injections. Animal was moribund.

bloods is not stated. Inspection of the data, however, shows that the same control values appear for several groups of similarly treated animals. This might be due to coincidence, or the use of one set of controls for several groups. If it be true that control values were taken from one pair of animals and experimental values from another pair, the significance of the difference found by Riddle and Dotti would be seriously impaired. In the light of normal variation, a difference of 1.5 to 3.1 mg. per cent in serum calcium between 2 groups of 2 animals each does not appear to be of great significance.

Increases in serum calcium as great as 16.1 mg. per cent (from 9.0 to 25.1) are reported in pigeons and doves by Riddle and Dotti. Such increases are obviously similar to that occurring with the deposition of the calcium-rich shell (7). Since no calcareous shell formation occurs in mammals, it seems unjustifiable to infer that a similar rise would occur in them. As regards this response data obtained from the bird are not applicable to mammalian physiology. This is attested by the slight and probably insignificant rise reported in the few rats and 2 dogs (no rise was secured in rabbits) injected by Riddle and Dotti (3), and by the data from our work and that of Marlow and Koch (2) and others (8, 9). Other investigators (10, 11) have even claimed de-

crease in serum calcium as a result of administration of crude estrogenic preparations.

We seriously doubt whether estrin actually has a physiologically significant effect on the mammalian blood calcium level. Our own data, as well as those of Riddle and of others (2, 3, 8, 9; for further references see 2) show mean changes of such small magnitude as to make one skeptical, even though some of these increases appear statistically significant. Furthermore, it appears from our data that in those cases where the huge doses of estrin did have a statistically significant effect, such change was only transitory. The serum calcium returned to normal values if treatment was continued. Therefore implications concerning the influence of estrin, via the calcium balance, on growth, stature, bone formation, etc., seem of doubtful significance.

An interesting point is the relative constancy shown by the serum calcium level of our rabbits. The data of Marlow and Koch (2) show great variation in the serum calcium of rabbits (9.1 to 16.0 mg. per cent) as well as rats (4.7 to 11.1 mg. per cent). Dixon (9), Dupré and Semenoff (12) and Bourne and Campbell (13) all report considerable fluctuation of the rabbit serum calcium level, unless the animals are fasted 12 to 24 hours prior to blood withdrawal. Our data for rabbits kept on a diet of oats, hay and lettuce show no significant change after 24 hours' fasting. Others (11, 14, 15) have also found constant serum calcium values for rabbits.

SUMMARY

The serum calcium levels of normal and ovariectomized adult female rats and rabbits and of normal immature monkeys were determined before and after administration of high doses of theelin or dihydrotheelin. The data, derived from 143 calcium determinations made on the sera of 44 rats, 19 rabbits and 6 monkeys, indicate very little if any effect of estrin administration or ovariectomy on the serum calcium level. Such slight elevation as was noted after estrin treatment appears to be only transitory in nature.

REFERENCES

1. Zwarenstein, H.: Biol. Rev. **9**:299. 1934.
2. Marlow, H. W., and F. C. Koch: Endocrinology **21**:72. 1937.
3. Riddle, O., and L. B. Dotti: Science **84**:557. 1936.
4. Saviano, M.: Mem. R. Acad. Naz. Lin., Sci. Fis. **6**:165. 1935.
5. Clark, E. P., and J. B. Collip: J. Biol. Chem. **64**:461 1925.
6. Fisher, R. A.: Statistical Methods for Research Workers. Oliver & Boyd, Edinburgh, 1934.
7. Riddle, O., and W. H. Reinhardt: Am. J. Physiol. **76**:660. 1926.
8. Mathieu, F., and B. O. Barnes: Am. J. Physiol. **105**:172. 1933.
9. Dixon, T. F.: Biochem. J. **27**:410. 1933.
10. Mirvish, L., and L. P. Bosman: Quart. J. Exper. Physiol. **18**:11. 1927.
11. Reiss, M., and K. Marx: Endokrinologie **1**:181. 1928.
12. Dupré, E. P., and E. Semeonoff: J. Biol. Chem. **94**:341. 1931.
13. Bourne, M. C., and D. A. Campbell: Biochem. J. **26**:183. 1932.
14. Hogben, L., and E. Charles: J. Exper. Biol. **9**:139. 1932.
15. Cannavo, L.: Biochem. Ztschr. **245**:234. 1932.

OBSERVATIONS ON THE QUANTITATIVE ASSAY OF GROWTH-PROMOTING EXTRACT OF THE HYPOPHYSIS

C. CHOU, CHIN CHANG, GRAHAM CHEN, AND H. B. VAN DYKE
From the Department of Pharmacology, Peiping Union Medical College
PEIPING, CHINA

The assay of the growth-promoting principle of the anterior pituitary has received attention in only a few reports. These have been reviewed by van Dyke (1), and since his review was written no new data specifically bearing on this problem have been published. The purpose of our communication is to record attempts quantitatively to assay the hormone in normal and hypophysectomized rats.

METHODS

All the experiments were performed with a single growth-promoting extract made from powdered beef anterior lobe which had been defatted and desiccated by acetone. The purified solid extract was kept in a vacuum desiccator over P_2O_5. A sufficient amount of the extract was dissolved in water alkalinized to pH 7.4-7.8 in the highest concentration to be used. Appropriate dilutions were then made for other dose-levels, so that all doses were administered in a volume of 0.5 cc. per 100 gm. body weight, except in the series of 1936 when the volume administered was 1.0 cc. per 100 gm. body weight. Fresh solutions for all the groups of a given course were made up simultaneously every 3 days, and when not in use they were kept in a refrigerator. All injections were made intraperitoneally.

The rats used for assay, except those of the hypophysectomized group, were mature females from one inbred stock originally obtained from the Wistar Institute. The groups of approximately 20 rats each were made uniform in age and weight as far as possible. The initial mean weight (table 1) of the 1935 groups was 303.5 gm.; that of the 1936 group 257.7 gm. All the rats were treated alike (diet, time of feeding, etc.) except for variations in the dose of the growth-promoting extract. All were injected and weighed at the same time each day (1935 group: 86 rats; 1936 group: 125 rats). In 1935, the effect of the extract on 32 rats 12 to 15 months old was observed as also the effect in a preliminary experiment with 27 younger rats. A second course of injections was given to the 1935 groups after an interval of 6 weeks, for which purpose new groups were formed without reference to the previous doses.

The Use of the Normal Rat for Assay

The results of the experiment with normal adult female rats are tabulated

in table 1. In an effort to evaluate the significance of the results, a number of observations were compared by Fisher's method; these comparisons, given in the appendix of table 1, indicate the probability that similar differences might be found if dose-variation had not been introduced. Much of the following discussion is based on these data (table 1).

How accurately can the growth-promoting hormone be assayed in normal adult female rats? Despite the use of fairly large groups of rats, and despite all efforts to introduce only one variable, i.e., the dose, the experimental results were not quantitatively satisfactory. In the groups receiving the first course in 1935, an increase of 100 per cent in the dose (from 1 to 2 mg. or from 2 to 4 mg.) at any time during the 20-day period was not followed by a significant increase in the body weight ($P = 0.05$ or less). When, however, the dose was quadrupled (from 1 to 4 mg.), the average body weight rose significantly after the tenth day, but not after the fifth day of injection. The results of the first course of 1936 indicate that a difference of 100 per cent in the dose may sometimes cause a significant difference in the body weight even after the fifth day (1 and 2, and 2 and 4 mg. doses). A 50 per cent change in dose brought about no significant change in weight (2 and 3 mg., and 4 and 6 mg.), nor did 100 per cent change at a higher dose level (3 and 6 mg.). It is also noteworthy that the growth-promoting effect of the 6 mg. dose in the 1936 series was less—but not significantly less—than that of the 4 mg. dose. Perhaps the better discrimination obtained in the 1936 series also depended upon the use of younger female rats. These results suggest that a change in dose amounting to 100 per cent may produce a clear-cut change in body weight, provided the smaller dose causes only a small or moderate weight increase, even after injections have been continued for 20 days (5 to 13 per cent compared with 2.5 per cent in uninjected controls).

How long should injections be continued? The data in table 1 indicate that a significant change in body weight, probably attributable to dose, appears after injections have been repeated for 10 days, and that a 20-day injection period is not accompanied by a worth-while improvement in results. In every instance except one (2 and 4 mg., 1936 course) in which dose-variation caused a significant change in body weight, this change was evident after 10 days. In about 30 per cent of all the groups receiving injections the weight increase after the 20th day of injections was not significantly greater than that after the 10th day. Likewise, approximately 60 per cent of the total change in weight in the 20-day injection period had appeared by the 10th day (table 6). Of course, the rate of change in weight fell even more.

The desirability of an injection period of 10, in preference to 20, days is further shown by comparing the responses of rats to the first courses of 1935 and 1936, respectively. In every instance, a significant difference appeared at the end of 10 days' injection ($P = 0$). At the higher dosage levels (2 and 4 mg.), however, the difference was not significant at the end of 20 days ($P = 0.09$ and 0.07).

The response to a second course of injections. The second course of in-

TABLE 1. THE EFFECT OF GROWTH-PROMOTING EXTRACT ON THE WEIGHT OF NORMAL ADULT FEMALE RATS

Course	No. of rats	Dose, mg. per day per 100 gm. body weight	Mean and *S.E.* of percentage change in weight (in terms of pre-injection weights) after injections for			
			5 days	10 days	15 days	20 days
1935 First	24	0	0.94[1]	3.63	4.44	4.96
1935 Second	20	0	(1) 1.96±0.49	1.40±0.59	0.89±0.64	0.34±0.70
1936 First	21	0	(2) 0.65±0.30	(15) 1.04±0.43	(30) 1.40±0.46	(45) 2.47±0.47
1935[2]	8	0	(3) 0.09±0.80	−0.78±0.58	−2.32±0.69	−2.92±0.69
1935 First	20	1	4.48[1]	(16) 8.40±0.73	(31) 7.84±0.80	(46) 11.00±1.23
1935 Second	20	1	(4) 3.02±0.35	(17) 4.75±0.63	(32) 5.46±0.73	(47) 6.38±0.82
1936 First	22	1	(5) 1.00±0.26	(18) 2.97±0.38	(33) 3.65±0.62	(48) 4.82±0.60
1935[2]	8	1	(6) 3.68±0.82	(19) 3.72±1.09	(34) 3.16±1.27	(49) 3.64±1.42
1935 First	21	2	4.76[1]	(20) 9.76±0.81	(35) 10.29±1.13	(50) 12.51±1.38
1935 Second	19	2	(7) 4.81±0.53	(21) 6.48±0.78	(36) 8.86±0.81	(51) 9.67±0.77
1936 First	21	2	(8) 2.30±0.37	(22) 4.96±0.53	(37) 7.40±0.65	(52) 9.96±0.64
1935[2]	8	2	(9) 4.88±0.85	(23) 7.56±1.61	(38) 7.71±1.84	(53) 7.81±2.22
1936 First	20	3	(10) 2.27±0.32	(24) 5.23±0.54	(39) 8.32±0.69	(54) 12.10±0.94
1935 First	21	4	3.27[1]	(25) 11.58±0.85	(40) 12.23±0.87	(55) 15.56±0.90
1936 First	21	4	(11) 2.46±0.46	(26) 6.06±0.59	(41) 9.42±0.67	(56) 13.28±0.80
1935 Second	20	5	(12) 4.91±0.57	(27) 7.87±0.73	(42) 11.11±1.12	(57) 13.18±1.30
1935[2]	8	5	(13) 4.60±0.79	(28) 6.56±0.85	(43) 9.13±0.79	(58) 9.70±1.34
1936 First	20	6	(14) 2.57±0.43	(29) 5.43±0.50	(44) 8.89±0.60	(59) 12.27±0.74

[1] Failure to include the *S.E.* is due to accidental loss of detailed data.
[2] Groups of rats 12 to 15 months old.

TABLE 1 (APPENDIX)

Observations compared[1]	P[2]	Observations compared[1]	P[2]	Observations compared[1]	P[2]
1,4	0.09	20,23	0.19	36,51	0.48
3,6	0.045	20,25	0.13	37,39	0.32
4,7	0.005	20,50	0.09	37,41	0.03
4,17	0.016	21,36	0.035	37,44	0.09
4,32	0.002	21,51	0.004	40,41	0.01
4,47	0.0002	22,26	0.16	40,43	0.04
5,8	0.004	22,29	0.55	40,55	0.009
7,21	0.07	25,26	0	42,57	0.23
7,36	0.00002	25,27	0.001	45,48	0.003
7,51	0	25,28	0.001	46,47	0.002
12,27	0.001	25,55	0.001	46,48	0.0001
13,28	0.18	27,42	0.016	46,50	0.42
13,43	0.004	27,57	0.0003	46,55	0.002
15,18	0.001	28,43	0.08	47,51	0.003
16,17	0.0001	28,58	0.11	50,51	0.0001
16,18	0	30,33	0.005	50,52	0.09
16,19	0.001	31,32	0.027	50,53	0.08
16,20	0.23	31,33	0.00003	50,55	0.07
16,25	0.005	31,34	0.003	52,54	0.06
16,46	0.07	31,35	0.09	52,56	0.001
17,21	0.09	31,46	0.03	52,59	0.02
17,47	0.11	32,36	0.002	54,55	0.007
18,22	0.002	35,36	0.31	55,56	0.07
18,24	0.0005	35,37	0.03	55,57	0.13
20,21	0.003	35,38	0.25	55,58	0.0008
20,22	0	35,40	0.16		

[1] The numbers refer to those given in parentheses in table I.
[2] Probability that as great a difference would be encountered in random observations of the effect of one dose.

jections in 1935 was begun 6 weeks after the completion of the first. Almost always a significantly lessened weight change followed a second course (table 1 and appendix). This may have been due to 'antihormone', diminished growth potentiality, etc. The data of table 2 indicate that it was probably not the result of deterioration of the extract, and also suggest that the greater the dose of the first course the less will be the response to the second course, especially if the dose of the latter is small.

Age as a factor in the response to growth-promoting extract. If the growth response of rats 12 to 15 months old is compared with that of the

TABLE 2. THE EFFECT OF PREVIOUS ADMINISTRATION OF GROWTH-PROMOTING EXTRACT ON THE RESPONSE TO A SECOND COURSE OF INJECTIONS

Dose of first course in mg.	Mean and *S. E.* of percentage gain in weight after second course								
	N		Dose: 1 mg.	N		Dose: 2 mg.	N		Dose: 5 mg.
0	4	A	12.92±1.84	12	E	11.08±0.98	12	G	13.64±1.04
1	4	B	7.55±1.29	4		9.25±2.72	4		9.72±2.80
2	3	C	3.00±2.44	6		7.13±2.58	7		10.30±1.89
4	6	D	2.33±1.51	5	F	6.92±2.11	5	H	7.02±1.46
			P			*P*			*P*
Groups compared	AB		0.05	EF		0.06	GH		0.003
	AC		0.02						
	AD		0.004						

The effect of repeated courses to the same animals

Course of injections	N	Dose: 1 mg.	N	Dose: 2 mg.	N	Doses: 4 mg.–5 mg.
First	4	12.92±1.84	6	10.57±2.63	5	14.18±1.85
Second		7.55±1.29		7.13±2.58		7.02±1.46
P		0.05		0.36		0.01

All doses expressed as mg. per 100 gm. body weight per day for 20 days.
N = Number of animals. *P* = As in appendix of table 1.

younger adult female rats likewise receiving the extract for the first time (1935, first course), it is clear that the response was always less in the rats of an age of 1 year or more. Moreover, it was significantly less, except in the groups receiving a dose of 2 mg. It must be realized, however, that control rats 12 to 15 months old exhibited even a loss of weight during the injection period, whereas the younger rats maintained their weight, or gained slightly.

Deterioration of the extract. Although the extract was kept as a dry powder over P_2O_5 in a desiccator, it apparently lost considerable activity in a year. The first course of 1936 was given to new groups of younger but otherwise similar rats under similar conditions one year after the second course of 1935. The response was unquestionably less in the 1936 course, probably because of deterioration of the extract. A further test again made with 45 fresh animals 18 months after the first course of 1935 indicated that the extract had no effect in doses of 0.75 to 1.25 mg. per 100 gm. body weight.

The Use of the Hypophysectomized Rat for Assay

Rats 4 to 6 weeks old and weighing usually about 100 gm. (80 to 120 gm.) were hypophysectomized by a parapharyngeal approach similar to that of Smith (2). A test series of 130 completely hypophysectomized rats, 29 partially hypophysectomized rats, and 58 normal littermate control animals was used. The group of 29 partially hypophysectomized rats was, of course, heterogeneous. Most of these animals gained 2 to 3 gm. in weight each week for some weeks postoperatively. However, they soon reached a plateau above which there was no spontaneous increase. A few grew more rapidly, but even after several months were half (males) or two-thirds (females) the weight of control animals. On the basis of Smith's later report (3), one would conclude that 10 to 20 per cent of the pars glandularis had not been removed. No spontaneous growth occurred in the completely hypophysectomized rats.

To determine the effect of age on the response of hypophysectomized rats to the growth-promoting extract, operations were performed at intervals so that 3 groups of animals were available. In these groups there had been a complete or partial hypophysial deficiency for 8 to 10, 4 to 6, and about 1 month(s), respectively. The age of each group was 1 to 1.5 months greater. Whether hypophysectomized or normal, each animal received a course of injections only once.[1] Daily weighings and injections were undertaken exactly as in the experiments with adult female rats. The results summarized in tables 3 and 4, column 6, represent the effects of injections continued for 20 days.

Comparisons between normal, partially hypophysectomized and completely hypophysectomized rats (table 3). Although it is now generally agreed that hypophysectomized rats are more sensitive to the growth-promoting effects of suitable anterior pituitary extracts, few accurate comparative data have been published. All the comparisons in the 3 divisions of table 3 were made with littermate rats which received the same extract under conditions as rigidly uniform as possible (injections, weighing, feeding, etc., at the same time each day). There can be no doubt that completely hypophysectomized male or female rats with 4 to 6 or 8 to 10 months' hypophysial deficiency exhibited a much greater response to the growth-promoting extract than did littermate normal rats. A similar clear-cut but less striking difference was shown by comparing groups of partially hypophysectomized and normal rats (division 2, table 3). Indeed, the response of partially hypophysectomized rats was intermediate between that of normal and that of completely hypophysectomized animals. Division 3 of table 3 shows that the increase in body weight following the injection of growth-promoting hormone is significantly greater after complete hypophysectomy than after partial hypophysectomy in 3 of the 4 comparisons.

The effect of the duration of hypophysial deficiency on the response to growth-promoting extract. Inasmuch as old normal female rats do not respond to injections of growth-promoting extracts as well as young adult females, it is not surprising that rats suffering from a long period of hypophysial deficiency before injection should respond less satisfactorily than those whose period of deficiency is shorter (division 1, table 4). The injection of growth-

[1] Except 1 group of hypophysectomized male rats. See tables 5 and 6.

TABLE 3. COMPARISON OF THE RESPONSE OF LITTERMATE NORMAL AND PARTIALLY OR COMPLETELY HYPOPHYSECTOMIZED RATS

Group	N	Sex	Months after operation	Dose (mg.)	Mean and *S. E.* of percentage increase in body weight	*P*
1 c	4	♂	8–10	0.5	15.54±0.58	<0.000001
1 n	4	♂		0.5	1.90±0.53	
2 c	19	♂	4– 6	0.5	23.31±1.57	<0.000001
2 n	22	♂		0.5	1.97±0.92	
1 c	11	♀	8–10	0.5	18.34±1.46	0.012
1 n	5	♀		0.5	9.23±1.52	
2 c	18	♀	4– 6	0.5	23.73±1.64	<0.000001
2 n	27	♀		0.5	9.77±0.58	
1 p	2	♂	8–10	0.5	6.09±0.85	0.012
1 n	4	♂		0.5	1.90±0.53	
2 p	9	♂	4– 6	0.5	10.51±1.47	0.000001
2 n	22	♂		0.5	1.97±0.92	
2 p	15	♀	4– 6	0.5	14.46±1.56	0.0009
2 n	27	♀		0.5	9.77±0.58	
1 c	4	♂	8–10	0.5	15.54±0.58	<0.004
1 p	2	♂		0.5	6.09±0.85	
2 c	19	♂	4– 6	0.5	23.31±1.57	<0.000001
2 p	9	♂		0.5	10.51±1.47	
1 c	11	♀	8–10	0.5	18.34±1.46	0.23
1 p	13	♀		0.5	13.44±1.05	
2 c	18	♀	4– 6	0.5	23.73±1.64	0.0007
2 p	15	♀		0.5	14.46±1.56	

N=Number of animals. c=Completely hypophysectomized rats. p=Partially hypophysectomized rats. n=Normal littermate. *P*=Probability that as great a difference would be encountered in random experiments if the groups were alike.

promoting extract into male or female animals hypophysectomized 8 to 10 months before injection did not cause as great an increase in weight as in those with a complete hypophysial deficiency of 4 to 6 months or less. Nevertheless, rats of both sexes with a hypophysial deficiency of 8 to 10 months' duration still responded better to growth-promoting extract than normal adult females. On the other hand, the difference in response was not significant in rats injected after the pituitary deficiency had been present for 4 to 6 months, compared with one month.

Smith (2) concluded that the life-span of the hypophysectomized rat was perhaps less than one-half that of the normal rat. He refers to 2 rats, operated at ages comparable to those of our rats, which survived to the ages of 10 and 15.5 months, although they were kept under especially favorable conditions. All of our completely hypophysectomized rats were kept under the same conditions as the normal rats and survived to an average age of 18 months (14 to 25 months).

The lack of a sexual difference in response of hypophysectomized rats (division 2, table 4). Confirming the reports of Evans and Simpson (4) and

Simon and Binder (5), we found that the growth-promoting effect of an extract could be demonstrated much more clearly in normal female rats than in normal males. In a few of the partially hypophysectomized rats, a similar sexual difference in response was observed. In completely hypophysectomized rats, however, the response was the same in both males and females. This dis-

TABLE 4. THE EFFECT OF GROWTH-PROMOTING EXTRACT ON THE WEIGHT OF HYPOPHYSECTOMIZED RATS AND THEIR NORMAL LITTERMATES

Group	No. of rats	Sex	Months after operation	Dose	Mean and *S. E.* of percentage increase in body weight	*P*
I. The effect of the duration of hypophysial deficiency						
1 c	4	♂	8–10	0.5	15.54±0.58	0.028
2 c	19	♂	4– 6	0.5	23.31±1.57	
1 c	11	♀	8–10	0.5	18.34±1.46	0.036
2 c	18	♀	4– 6	0.5	23.73±1.64	
1 c	11	♀	8–10	0.5	18.34±1.46	0.0037
3 c	14	♀	1	0.5	26.75±2.04	
2 c	18	♀	4– 6	0.5	23.73±1.64	0.25
3 c	14	♀	1	0.5	26.75±2.04	
II. The effect of difference in sex in normal and hypophysectomized rats						
1 c	4	♂	8–10	0.5	15.54±0.58	0.43
1 c	11	♀	8–10	0.5	18.34±1.46	
2 c	19	♂	4– 6	0.5	23.31±1.57	1.00
2 c	18	♀	4– 6	0.5	23.73±1.64	
2 c	20	♂	4– 6	0.25	17.46±1.56	0.37
2 c	18	♀	4– 6	0.25	15.80±0.96	
1 p	2	♂	8–10	0.5	6.09±0.85	0.01
1 p	3	♀	8–10	0.5	13.44±1.05	
2 p	9	♂	4– 6	0.5	10.51±1.47	0.045
2 p	15	♀	4– 6	0.5	14.46±1.56	
1 n	4	♂	8–10[1]	0.5	1.90±0.53	0.004
1 n	5	♀	8–10[1]	0.5	9.23±1.52	
2 n	22	♂	4– 6[1]	0.5	1.97±0.92	<0.000001
2 n	27	♀	4– 6[1]	0.5	9.77±0.58	
III. The effect of variation of dose						
2 c	20	♂	4– 6	0.25	17.46±1.56	0.0019
2 c	19	♂	4– 6	0.5	23.31±1.57	
2 c	18	♀	4– 6	0.25	15.80±0.96	<0.000001
2 c	18	♀	4– 6	0.5	23.73±1.64	
3 c	11	♀	1	0.25	16.42±1.99	0.0005
3 c	14	♀	1	0.5	26.75±2.04	

c=Completely hypophysectomized rats. p=Partially hypophysectomized rats. n=Normal littermates. *P*=Probability that as great a difference would be encountered in random experiments if the groups were alike or had been treated alike.

[1] Age-groups.

appearance of a sexual difference in sensitivity following hypophysectomy cannot be attributed to a loss or lack of the internal secretions of the gonads, inasmuch as Evans and Simpson showed that the castrated rat, although more responsive than the normal male, still remains less sensitive than the spayed or even the normal female.

The effect of variation of the dose of growth-promoting extract administered to hypophysectomized rats (division 3, table 4). It will be recalled that an increase of 100 per cent in the dose of the growth-promoting extract might (1936 courses: 1 and 2, and 2 and 4 mg.) or might not (1935 courses: 1 and 2, and 2 and 4 mg.; 1936 courses: 3 and 6 mg.) bring about a significant difference in response. In all cases of hypophysectomized rats, a difference of 100 per cent in dose (0.25 and 0.5 mg.) caused a convincing difference in response. The absolute difference in the dose was of course only one-fourth (or less) that used in normal female rats.

It is possible that a smaller percentage difference in dose could have been clearly detected in hypophysectomized rats at higher or lower dosage levels, or with doses between 0.25 and 0.5 mg. From data selected at random, we have also investigated the feasibility of using smaller groups of hypophysectomized rats for determining a difference in the effects of the doses 0.25 and 0.50 mg. Only infrequently (4 of 13 comparisons) was there a probable difference in effect ($P = 0.02$-0.05), when 5 to 6 rats were used. If 9 rats were used in each group, a difference could be shown in 5 of 8 comparisons ($P = 0.005$-0.03). In all comparisons, with groups of 15 rats, a significant difference in response could be shown ($P = 0.001$-0.035). The results were about the same for injection periods of 10 and 20 days.

How long should injections be continued? The effect of a second course of injections. A qualitative test for the presence of growth-promoting hormone can be satisfactorily completed in the hypophysectomized rat after only a few days. Analysis of the data of table 4, section 3, indicated that the dose variation used was sometimes followed by a significant variation in the weight response, after injections had been repeated for 5 days. After an injection period of 10 days, there was always a significant difference in weight response ($P = 0.0007$ or less). Therefore, our data indicate that injections of growth-promoting extract into completely hypophysectomized rats for purposes of assay should not be continued longer than 10 days. In fact, differences in response after 20 days' injection may be less striking than after injections have been continued for only 10 days.

A group of 15 male hypophysectomized rats was given the extract for 1 week in a preliminary experiment 2 weeks before the injection of all the hypophysectomized groups (see tables 5 and 6). This group also received the extract (dose: 0.5 mg.) simultaneously with the others. The same dose administered to hypophysectomized female rats of the same age produced a significantly greater weight response even after 5 days ($P = 0.00002$). The response in similar female rats receiving 0.25 mg. of the extract was usually less, but at no time during the 20 days significantly less than that of the group of 15 males. The response of male hypophysectomized rats, 4 to 6 months after

TABLE 5. THE EFFECT OF A PREVIOUS INJECTION OF GROWTH-PROMOTING EXTRACT ON THE RESPONSE OF A GROUP OF HYPOPHYSECTOMIZED RATS

N[1]	Sex	Months after operation	Dose, mg. day per 100 gm. body weight	Mean and *S. E.* of percentage change in weight (in terms of pre-injection weights) after injections for			
				5 days	10 days	15 days	20 days
15[2]	♂	1	0.50	5.00±0.74	12.48±1.21	15.66±1.48	18.83±1.66
11[3]	♀	1	0.25	6.91±0.68	10.72±1.01	13.91±1.94	16.42±1.99
14[3]	♀	1	0.50	8.48±0.67	16.17±1.18	21.27±1.88	26.75±2.04
19[3]	♂	4–6	0.50	8.89±0.62	14.14±0.92	19.16±1.15	23.31±1.57

[1] Number of animals.
[2] Received growth-promoting extract in a dose of 0.5 mg. for 7 days previously. A rest-period of 2 weeks intervened.
[3] No previous injection.

TABLE 6. THE MEAN CHANGE IN WEIGHT OF VARIOUS GROUPS OF RATS AT 5-DAY INTERVALS THROUGHOUT THE 20-DAY PERIOD OF INJECTION OF GROWTH-PROMOTING EXTRACT

Group		N[1]	Months after operation	Dose, mg. per day per 100 gm. body weight	Mean gains in successive 5-day periods							
					Absolute (gm.)				Percentage change in terms of pre-injection weights			
Hypophysectomized	♂	4	8–10	0.50	4.25	5.75	4.00	5.25	3.45	4.68	3.25	4.16
		20	4– 6	0.25	7.00	4.00	4.35	2.60	6.52	3.73	4.05	3.16
		19	4– 6	0.50	9.11	5.36	5.16	4.16	8.89	5.25	5.02	4.15
		15[2]	1	0.50	5.53	8.34	3.53	3.67	5.00	7.48	3.18	3.17
	♀	11	8–10	0.50	5.09	5.09	4.09	5.91	4.58	4.58	3.69	5.49
		18	4– 6	0.25	6.61	3.00	2.15	3.79	6.69	3.04	2.19	3.88
		18	4– 6	0.50	8.89	5.94	3.95	2.83	9.73	6.50	4.32	3.18
		11	1	0.25	6.09	3.37	2.81	2.00	6.91	3.81	3.19	2.51
		14	1	0.50	7.81	6.90	4.65	5.07	8.48	7.69	6.10	5.48
								Sum	60.25	46.76	33.99	35.18
Normal adult ♀ (first series)		27[3]		0.50	11.63	6.04	3.66	3.23	4.59	2.39	1.45	1.27
		20		1.00	13.43	11.74	−1.68	9.47	4.48	3.92	−0.56	3.16
		21		2.00	14.88	15.63	1.66	6.94	4.76	5.00	0.53	2.22
		21		4.00	10.05	25.53	2.00	10.23	3.27	8.31	0.65	3.33
Normal adult ♀ (new series one year later)		22		1.00	2.50	4.91	2.22	6.39	1.00	1.97	0.68	1.17
		21		2.00	5.86	6.76	6.38	10.96	2.30	2.66	2.44	2.56
		20		3.00	5.70	7.50	7.95	9.10	2.27	2.96	3.09	3.78
		21		4.00	6.29	9.28	9.15	10.14	2.46	3.60	3.36	3.86
		20		6.00	6.80	7.80	9.03	8.82	2.57	2.86	3.46	3.38
								Sum	27.70	33.67	15.10	24.73

[1] Number of animals.
[2] This group had previously received a short course of injections of growth-promoting extract in a dose of 0.5 mg.
[3] Not injected simultaneously with the other groups of this series.

hypophysectomy, was also compared with that of the recently operated male rats which had previously received a short course of injections. The response of the older male rats was always greater, although the difference was not significant ($P = 0.27, 0.06, 0.06$) except after 5 days' injection ($P = 0.00006$). It may be concluded, therefore, that even a short course of injections of growth-promoting extract followed by a rest period of 2 weeks may reduce the sensitivity of the hypophysectomized rat.

Other Comparisons Between the Group-Responses of Completely Hypophysectomized Rats and Normal Adult Females

The mean change in weight at 5-day intervals. The mean changes in weight of rats of a number of groups are summarized in table 6, and both the absolute and the relative gains are given. In the hypophysectomized as well as the normal adult female groups, about 60 per cent of the total relative weight increase occurred after injections had been repeated for 10 days. In the hypophysectomized groups, the greatest change occurred during the first 5 days (34.5 per cent); in the groups of normal adult females, the greatest change was observed in the second 5-day period (33.3 per cent).

TABLE 7. THE LOSS OF WEIGHT OF HYPOPHYSECTOMIZED AND NORMAL RATS 1 WEEK AFTER THE LAST INJECTION OF GROWTH-PROMOTING EXTRACT

Group		Months after operation	Daily dose mg./100 gm.	No. of rats	A Gain in 20 days (Percentage of pre-injection weight)	Loss in 1 week (Percentage of A)
Hypophysectomized	♂	4– 6	0.25	20	17.46±1.56	13.40±4.12
		4– 6	0.5	19	23.31±1.57	20.58±5.04
		1[1]	0.5	15	18.83±1.66	38.92±5.38
	♀	8–10	0.5	11	18.34±1.46	27.50±4.21
		4– 6	0.25	18	15.80±0.96	29.29±4.61
		4– 6	0.5	18	23.73±1.64	26.21±4.69
		1	0.25	11	16.42±1.99	29.85±7.61
		1	0.5	14	26.75±2.04	19.85±4.38
Normal adult ♀ (First series)			1	20	11.00±1.23	24.39±5.85
			2	21	12.51±1.38	16.65±6.55
			4	21	15.56±0.90	23.59±3.79
Normal adult ♀ (Second series one year later)			3	20	12.10±0.94	33.22±6.63[2]
			4	21	13.28±0.80	34.42±4.96[2]
			6	20	12.27±0.74	32.66±5.22[2]

[1] This group had received a short course of injections of growth-promoting extract 2 weeks previously.
[2] Eight days after last injection.

The loss of weight following injection. The principal loss of weight following injection for a period of 20 days occurred during the first week of the post-injection period. Representative data on the extent of this post-injection loss can be found in table 7. The percentage of the total gain that was lost 7 to 8 days after an injection period of 20 days is given in the last column. The great variation in the extent of the loss seems unrelated to the previous gain or to the presence or absence of the pituitary.

DISCUSSION

The quantitative assay of growth-promoting extract is extremely difficult. That it may be partially simplified by the use of highly potent extracts is doubtful because of the ease with which these may undergo deterioration (Evans and others). The extract used in all our experiments, although still crude and preserved dry as a solid, underwent considerable deterioration in the course of a year. Assay is of course more convenient if normal rats can be used and for

such a purpose only female animals should be employed (4, 5). Different investigators using normal rats have repeated the injections for 3 to 20 days. Our results indicate that the optimum period is about 10 days. We do not believe that groups of 6 rats each, as used by Evans, Meyer, and Simpson (6), are sufficiently large for quantitative assay. Although at least 20 rats were included in each of our groups of normal rats, a dose-variation of 100 per cent could often not be clearly detected. As would be expected, differences in doses producing a moderate increase in weight appear to be more easily distinguished. Older female adults were less sensitive than young female adults. This finding is contrary to that of Evans and Simpson (4) who concluded that the response is not materially altered in old rats. However, their data indicate that the relative change in weight is diminished in older rats. Moreover, their data do not indicate that a maximal response was not obtained in the younger rats.

Studies on the assay of growth-promoting extract by the use of hypophysectomized rats have been made by van Dyke and Wallen-Lawrence (7), Collip, Selye, and Thomson (8), as well as by Evans, Pencharz, Simpson, and Meyer (9). The preparation used by van Dyke and Wallen-Lawrence produced about the same relative response in 2 groups of rats, one normal, the other hypophysectomized. Injections were made for only 3 days. The growth-response of the hypophysectomized group was remarkably small, probably because many of the rats had already received previous treatment with growth-promoting extract. Also the hypophysial deficiency was of long duration in some members of the group. Collip, Selye, and Thomson used hypophysectomized rats weighing about 100 gm. for the assay of growth-promoting extract. They recommended that such rats should be used only once, and administered the extract over a period of 15 days. In our opinion, a shorter period of injection (about 10 days) is preferable. Evans and his colleagues pointed out that hypophysectomized rats always appeared to be more responsive to growth-promoting extracts than normal rats.

The preparation of adequate groups of hypophysectomized rats requires time and considerable technical skill. However, the hypophysectomized rat, which has not received extract previously, is greatly superior to the normal rat for assay purposes. Male and female animals are equally sensitive. Spontaneous growth introduces no complicating factor which may require control groups. The sensitivity of hypophysectomized male or female rats, expressed as percentage increase in weight, is probably more than twice that of the normal young adult female. Likewise, variation in dosage is more easily recognized in assays performed with hypophysectomized animals. Partially hypophysectomized rats are less sensitive than completely hypophysectomized animals, and this is particularly true of male rats.

Van Dyke and Wallen-Lawrence found that some rats, previously responding to growth-promoting extract, failed several months later to respond to moderate doses. Age was the only factor considered important. It is probable, however, that the previous injection of extract had markedly reduced the sensitivity of the older hypophysectomized rats. Evans, Pencharz, Simpson, and

Meyer stated that old hypophysectomized rats respond well to growth hormone. We are in agreement with this statement provided that reference is made to animals which have not received extract previously. Evans and his co-workers also could "recognize no consistent difference in the reactivity of the hypophysectomized animals in different postoperative periods." Clearly their data were not sufficient to justify a conclusive statement.[2] However, our data suggest that rats with a hypophysial deficiency of 8 to 10 months are definitely less responsive than rats with a similar deficiency for 4 to 6 months or less, although more responsive than normal adult female rats. Statistically, there was no difference in the response of animals with hypophysial deficiencies of 4 to 6 months and 1 month.

Assays in either hypophysectomized or normal rats are rendered more difficult by the 'desensitizing' effect of a previous course of injections, although a rest period of 2 to 6 weeks intervenes. This difficulty, as well as some of the other difficulties already discussed, might be minimized by the simultaneous use of a standard preparation, provided this could be preserved without serious deterioration.

SUMMARY

Observations on quantitative aspects of the assay of the growth-promoting hormone of the pituitary were made in groups of rats totalling 288 normal adult females, 130 completely hypophysectomized rats (1 to 10 months' duration of hypophysial deficiency) together with 29 partially hypophysectomized rats and 58 littermate control animals. All the assays were performed with a single extract of the pars glandularis of the ox pituitary.

In terms of the percentage increase in weight, the sensitivity of the hypophysectomized rat is probably more than twice that of the normal adult female. Likewise, variation in dosage produces a clearer difference in response in the hypophysectomized rat. In the normal, unlike the hypophysectomized, rat, a dose variation of 100 per cent often fails to produce a convincing difference in response. There is no sexual difference in the sensitivity of the hypophysectomized rat. The normal female rat is more sensitive than the male.

The optimum period over which daily injections should be given is about 10 days or less. It appears to be the same in normal or hypophysectomized animals. The response of both normal and hypophysectomized rats after a rest period of 2 to 6 weeks was less if the extract had been given previously. The reduction in response appeared to be greater the larger the dose of the first course.

The loss of weight following injection is variable but similar in both normal and hypophysectomized rats.

REFERENCES

1. Van Dyke, H. B.: The Physiology and Pharmacology of the Pituitary Body, University of Chicago Press, Chicago, 1936.
2. Smith, P. E.: Amer. J. Anat. 45: 205. 1930.

[2] Some of the experiments of Evans and his co-workers indeed suggest that increasing age and duration of hypophysial deficiency do lessen the response to growth-promoting extracts (gain in 20 days with solutions M 1829 and M 2317). We assume that the same extract was used.

3. Smith, P. E.: Anat. Record, **52:**191. 1932.
4. Evans, H. M., and M. E. Simpson: Amer. J. Physiol. **98:**511. 1931.
5. Simon, A., and L. Binder: Arch. f. exper. u. Path. Pharmakol. **165:**120. 1932.
6. Evans, H. M., K. Meyer and M. E. Simpson: The Growth and Gonad-stimulating Hormones of the Anterior Hypophysis, p. 1, Univ. California Press, Berkeley, 1933.
7. Van Dyke, H. B., and Z. Wallen-Lawrence: J. Pharmacol. & Exper. Ther. **40:**413. 1930.
8. Collip, J. B., H. Selye and D. L. Thomson: Virchows Arch. f. path. Anat. **290:**23. 1933.
9. Evans, H. M., R. I. Pencharz, M. E. Simpson and K. Meyer: The Growth and Gonad-stimulating Hormones of the Anterior Hypophysis, p. 229, Univ. California Press, Berkeley, 1933.

THE BIOLOGICAL EFFECTS OF THYMUS EXTRACT (HANSON) ON THYMECTOMIZED RATS[1]

OVER-CORRECTION OF RETARDATION IN GROWTH AND DEVELOPMENT INCIDENT TO THYMECTOMY IN SUCCESSIVE GENERATIONS OF RATS

NATHAN H. EINHORN

From the Samuel Bell, Jr. Laboratory of the Philadelphia Institute for Medical Research, in the Philadelphia General Hospital

PHILADELPHIA, PENNSYLVANIA

It has been demonstrated by Rowntree, Clark, Steinberg and Hanson (1) that daily intraperitoneal injections of 1 cc. of a potent thymus extract into successive generations of albino rats of the Wistar strain results in an accruing acceleration in the rate of growth and development of the offspring. When it was found that thymectomy in successive generations of parent rats resulted in retarding the growth of their offspring, it seemed desirable to determine if this effect could be overcome by the administration of thymus extract (Hanson) to the thymectomized parent rats (2, 3).

METHOD OF EXPERIMENTAL PROCEDURE

Five pairs of albino rats (Wistar strain) of known parentage were selected from the normal stock animals of the Philadelphia Institute for Medical Research. Three pairs were thymectomized at the age of 21 days by the method described in an earlier communication, mated, and at 30 days started on daily intraperitoneal injections of 1 cc. of thymus extract (2). The remaining 2 pairs were subjected to the operative technique concerned in thymectomy, except that the thymi were not removed. These latter animals were designated operative controls. Data on somatic development were recorded daily and the weights of the rats twice weekly up to the age of 70 days. All of the animals in one litter or part of a large litter of each pair of parent animals were thymectomized, mated, and set up as parents of the next generation. Thus the offspring, in each generation, were young born to thymectomized parents receiving daily intraperitoneal injections of 1 cc. of thymus extract, the injections being started at the age of 30 days. All rats were housed and fed under the conditions previously described.

The thymus extract used in this experiment differed from the extract originally prepared by Hanson (1). The modified method was briefly as follows. One kilogram of neck thymus of freshly slaughtered 2 to 6 weeks-old

[1] Part of thesis studies submitted to the faculty of the Graduate School of Medicine of the University of Pennsylvania in partial fulfilment of the requirements for the degree of Doctor of Science, D.Sc. (Med.). This work was supported by a grant from the Penrose Fund of the American Philosophic Society and by the Rockefeller Foundation.

locally raised calves was ground in a meat-grinder. To this was added 1,000 cc. of 1 per cent (by volume) hydrochloric acid. The mixture was placed in a 4-liter flask, and extracted with the aid of heat and continuous stirring until the temperature reached 68°, when the flame was removed and the stirring discontinued. On standing, this mixture separated into two distinct layers consisting of a golden, clear, supernatant fluid and a flocculent tissue residue containing the protein. The filtrate was adjusted to a pH of 3.5 and bottled, whereupon it was ready for use. The extract was usually preserved by the addition of 0.2 per cent chlorbutanol and, thus prepared, 1 cc. represented 1 gm. of raw calf thymus gland.

TABLE 1. SOMATIC DEVELOPMENT IN SUCCESSIVE GENERATIONS OF THYMUS-TREATED THYMECTOMY GROUP

		Ears open	Upper incisors erupted	Hair appeared	Eyes open	Testes descended	Vagina open
Operative Controls	Range	2–3	7–10	11–14	13–17	24–35	39–50
	Average	2.9	8.5	12.5	15.2	30.2	44.3
F_1	Range	2–4	7–10	12–14	9–17	21–38	40–48
	Average	2.8	8.4	13.3	15.1	29.7	42.5
	Average, Thymectomy Strain[1]	3.4	9.4	14.8	15.9	30.4	45.1
F_2	Range	2–3	4–10	9–15	9–16	19–30	36–50
	Average	2.8	7.7	13.9	14.3	26.4	43.4
	Average, Thymectomy Strain[1]	3.3	9.5	14.2	16.2	30.7	52.0
F_3	Range	2–3	6–8	11–14	9–16	20–30	34–42
	Average	2.5	6.9	12.0	12.7	23.2	38.7
	Average, Thymectomy Strain[1]	3.2	9.6	13.6	16.3	30.4	46.5
F_4	Range	1–3	3–9	8–14	6–13	17–20	31–34
	Average	1.8	4.6	9.4	8.4	18.5	32.8
	Average, Thymectomy Strain[1]	3.5	9.6	14.8	16.8	30.0	48.8

All figures represent days.
[1] Untreated.

It has been found that biologically potent extracts prepared in this manner contain iodine-reducing substances in a concentration of at least 100 mg. per cent, and more recently the value has ranged from 150 to 220 mg. per cent, while biologically impotent extracts contain little or no demonstrable amount of iodine-reducing substances.

Data on operative control rats. A total of 208 animals was born from the second (F_1) to the fifth (F_4) generations of the original two pairs of operative controls (animals subjected to the technique concerned in thymectomy without actual removal of the thymus gland). Of this number, 183 or 87 per cent survived the 70-day period of observation. The rate of growth of these controls as reflected by the average weight curve is shown in figure 1, and the data on somatic development is given in table 1. As was pointed out in previous communications, the rate of growth and development in this special group of operative controls differed in no material way from that of the normal control series of the Philadelphia Institute for Medical Research (2, 3).

RESULTS OF CONTINUOUS ADMINISTRATION OF THYMUS EXTRACT TO THYMECTOMIZED RATS

Growth and development of test rats of the first generation (F_0). As a result of injecting 1 cc. of thymus extract daily into the original 3 pairs of thymectomized animals, no effect on their growth or behavior was noted. The average period between gestations was 37.9 days, as compared with 28.3 days

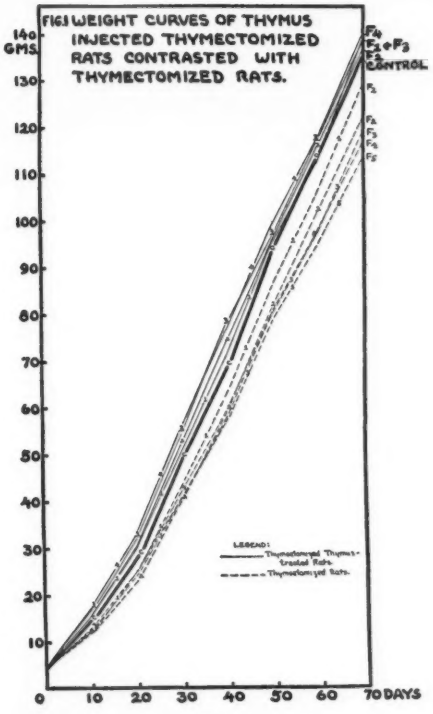

Fig. 1.

in the operative controls, and 37 days in the Philadelphia Institute series of control animals.

Second generation (F_1). A total of 169 rats was born in 18 litters to 3 pairs of thymus-treated thymectomized rats of the first generation (F_0). The litters varied in size from 7 to 12, with an average of 9.3 animals per litter. Of the total of 169 rats, 141, or 83 per cent survived the 70-day period of observation.

In the second generation (F_1) thymectomy group, a definite retardation in the rate of growth was noted (2, 3). In the offspring of the second generation (F_1) thymus-treated thymectomy group, this retardation in growth was not only completely offset but the average growth of the operative controls was exceeded by a slight margin throughout the entire period of observation (fig. 1 and table 2). In so far as somatic differentiation was concerned, a slight acceleration was observed in the opening of the ears, eruption of the upper in-

cisors, appearance of hair, opening of the eyes, descent of the testes, and opening of the vagina, which occurred often on the early normal day, and occasionally even earlier (table 1).

TABLE 2. AVERAGE WEIGHT IN GRAMS AT 10-DAY INTERVALS OF SUCCESSIVE GENERATIONS OF THYMUS-TREATED THYMECTOMY GROUP AND PERCENTAGE OF DIFFERENCE FROM OPERATIVE CONTROLS

Age, days	Operative Controls	F_1	% difference	F_2	% difference	F_3	% difference	F_4	% difference
Birth	4.9	4.9	0	5.0	2	5.0	2	5.0	2
10	15.0	15.6	4	15.8	5	17.0	13	17.2	15
20	28.5	30.0	5	30.5	7	32.0	12	32.6	14
30	49.1	50.5	3	52.6	7	54.4	11	55.0	12
40	68.6	70.7	3	74.0	8	77.6	13	76.8	12
50	93.5	94.9	1	95.8	2	96.7	3	98.2	5
60	113.3	113.5	0	115.8	2	116.7	3	116.7	3
70	135.3	138.0	2	136.8	1	138.0	2	139.4	3

Third generation (F_2). A total of 131 rats was born in 17 litters of the third generation (F_2). The litters varied in size from 5 to 9, with an average of 7.7 animals per litter. Of the total of 131 rats, 107, or 81 per cent survived the 70-day period of observation.

As in the second generation (F_1) thymus-treated thymectomy group, the retardation in growth resulting from thymectomy was completely offset (fig. 1 and table 2). It was evident that the rate of growth of the 131 rats of the third generation (F_2) not only exceeded the growth of the operative controls, but also the rate of growth of the second generation (F_1) by a small percentage. In addition to the acceleration in growth, precocious somatic development was noted in several instances (table 1). This precocity, however, was in no way as great as noted by Rowntree et al (1), in the third generation of the original thymus-treated test strain.

Fourth generation (F_3). A total of 166 rats was born in 20 litters of the fourth generation (F_3). The litters varied in size from 6 to 12, with an average of 8.3 animals per litter. Of the total of 166 rats, 144, or 86 per cent survived the 70-day period of observation.

As in the previous generations of the thymus-treated thymectomy strain, growth and somatic differentiation of these animals not only failed to show the retardation incident to thymectomy, but exceeded the growth and development of the operative controls and the animals of the second (F_1) and third generations (F_2), indicating an accumulation of effect in successive generations (fig. 1 and table 2). In two litters, the eyes opened on the ninth day, in one litter on the tenth day, and in 7 litters on the 12th day. The average for all of the animals of the fourth generation (F_3) was 12.7 days, as compared with 15.2 for the operative controls.

Fifth generation (F_4). A total of 154 rats was born in 20 litters of the fifth generation (F_4). The litters varied in size from 6 to 9, with an average of 7.7 animals per litter. Of the total of 154 rats, 123 had reached the end of the 70-day period of observation at the time of this writing.

As in the preceding generations of the thymus-treated thymectomy strain, the rate of growth as reflected by the average weight curve showed that the retardation in thymectomy had not only been completely overcome, but this growth exceeded by a small percentage that of the second (F_1), third (F_2), and fourth (F_3) generations, as is shown in figure 1 and table 2, again indicat-

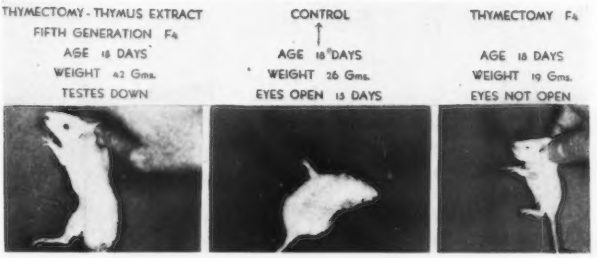

Fig. 2. Photographs showing over-correcting retardation in growth and development in thymectomized rats by means of substitution therapy with thymus extract.

ing an accumulation of effect in successive generations. Numerous instances of striking precocity were noted. In 7 litters, the upper incisors erupted on the fourth day; in 4 litters the eyes opened on the seventh day, and in 6 litters this occurred on the eighth day, thus indicating the relative frequency of marked acceleration in somatic differentiation (table 1).

EFFECTS OF THYMECTOMY WITH AND WITHOUT SUBSTITUTION THERAPY BY MEANS OF THYMUS EXTRACT (HANSON)

With reference to the ability of thymus extract in overcoming the retardation incident to thymectomy, it is of interest to compare the rate of growth and somatic differentiation, not only with that of the operative controls but with the retarded growth and development encountered in the thymectomy test strain. For convenience in making the comparison, there are shown in figure 1 the average growth curves of successive generations of the thymectomy and the thymus-treated thymectomy test strains. It is readily apparent that retardation in the rate of growth was present in the animals of the second generation (F_1) untreated strain, and that this retardation became more marked in each succeeding generation. On the other hand, this retardation was not only more than overcome in the second generation (F_1) thymus-treated thymectomized strain, but there can also be noted an increase in the rate of growth, which became progressively more marked in each succeeding generation.

The average retardation in growth of the fifth generation (F_4) thymectomy test strain was 6.9 gm., or 14 per cent at the age of 30 days. At the same age, the average acceleration in growth of the fifth generation (F_4) thymus-treated thymectomy strain was 5.9 gm., or 12 per cent. The difference between the average weights of these two groups of test animals at 30 days was 12.8 gm., or 26 per cent (fig. 2). Similarly, as compared with the untreated thymectomized animals, striking acceleration in somatic differentiation was noted in those treated. In the fifth generation (F_4) thymectomy test strain, the average

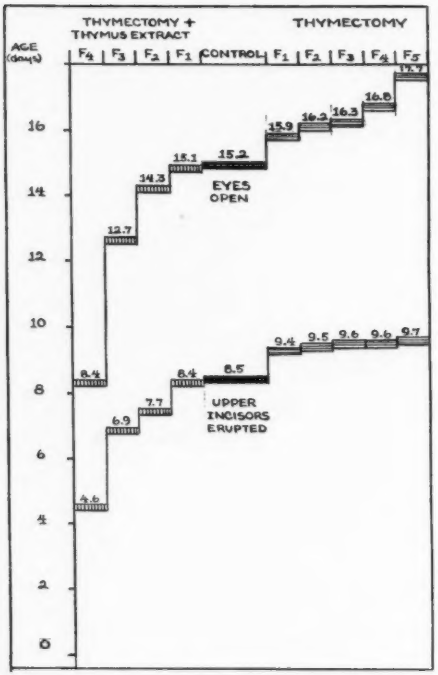

Fig. 3. Effect on somatic differentiation of treatment with thymus extract in successive generations of thymectomized rats contrasted with the effects in corresponding generations of untreated thymectomized rats.

time for the eruption of the upper incisors, and the opening of the eyes was 9.6 and 16.8 days, as compared with 4.6 and 8.4 days respectively in the thymus-treated thymectomy strain (fig. 2, 3). It would seem therefore that the daily intraperitoneal administration of 1 cc. of thymus extract has resulted in a marked over-correction of the retardation in growth and development incident to thymectomy.

DISCUSSION

This investigation was started on October 14, 1934, and at the time of this writing, February 1, 1937, had continued for over 27 months. The data reported have been obtained from observations made on a total of 834 rats, distributed in 5 generations as follows: operative controls 208, first generation (F_0) 6, second generation (F_1) 169, third generation (F_2) 131, fourth generation (F_3) 166, and fifth generation (F_4) 154.

In earlier communications, it was shown that removal of the thymus gland resulted in retardation in growth and development of the offspring (2, 3). The daily intraperitoneal administration of 1 cc. of thymus extract, prepared in the manner described, has not only completely overcome the retardation incident to thymectomy, but also steadily accelerated the growth and development in successive generations of thymus-treated thymectomized animals.

It is evident from the data reported in this communication that 1 cc. of thymus extract is more than the amount required to satisfy the daily needs of thymectomized rats. Just as there is a quantitative requirement for such substances as calcium and iodine, there may be a similar quantitative need for the principal agent or agents of the thymus gland. At the present time, quantitative experiments are being conducted to determine the daily need of rats of thymus expressed in terms of iodine-reducing substances.

SUMMARY

From observations made on 833 rats distributed in 5 generations, it appears that the accruing retardation in growth and development incident to thymectomy in successive generations of rats has not only been completely corrected, but has been changed to a moderate accruing acceleration in growth and development by daily intraperitoneal injections of 1 cc. of thymus extract (Hanson).

REFERENCES

1. Rowntree, L. G., J. H. Clark and A. M. Hanson: J.A.M.A. **103**:1425. 1934, *ibid.*, Tr. A. Am. Physicians **49**:252. 1935, *ibid.*, Arch. Int. Med. **56**:1. 1935.
Rowntree, L. G., J. H. Clark, A. Steinberg and N. H. Einhorn: Tr. A. Am. Physicians **51**:148. 1936.
2. Einhorn, N. H., and L. G. Rowntree: Endocrinology **20**:342. 1936.
3. Einhorn, N. H., and L. G. Rowntree: Science **83**:443. 1936.

THE BIOLOGICAL EFFECTS OF THYMUS IMPLANTATION IN NORMAL RATS[1]

ACCRUING ACCELERATION IN THE RATE OF GROWTH AND DEVELOPMENT IN SUCCESSIVE GENERATIONS OF RATS FOLLOWING FREQUENT HOMOLOGOUS THYMUS IMPLANTS[2]

NATHAN H. EINHORN AND LEONARD G. ROWNTREE
From the Samuel Bell, Jr. Laboratory of the Philadelphia Institute for Medical Research in the Philadelphia General Hospital
PHILADELPHIA, PENNSYLVANIA

After observing the increasing acceleration in the rate of growth and development through successive generations of rats subjected to the continuous injection of thymus extract (1), it was felt that the problem could also be approached by studying the effects of frequent homologous implants in two groups of animals: the first, a group from which the thymi had been surgically removed, and the second a group of normal stock animals. The results in the former experiment have been recorded in another communication.

METHOD OF EXPERIMENTAL PROCEDURE

Three pairs of albino rats (Wistar strain) of known parentage were selected from the normal stock animals of the Philadelphia Institute for Medical Research. These animals were mated, and started on weekly subcutaneous implants of 1 whole thymus gland obtained from stock rats to 20 to 30 days of age. The method of implantation has been described in another communication (2). Data on somatic development were recorded daily, and the weights of the rats twice weekly up to the age of 70 days. All of the animals in a small litter, or several of a large litter of each pair of parent animals, were mated and set up as parents of the next generation. Thus the offspring in each generation were young born to normal parent rats receiving frequent homologous thymus gland implants. Implantations were started at the age of 30 days, and a total of 12 implants was given to each parent animal. In the third generation (F_2), implantations were made at 5-day intervals with the hope of accentuating the effects obtained. It was found, however, that these animals became irritable and cast litters less frequently than when weekly implants were made, due possibly to interference with normal copulation because of the numerous painful areas on the back. All rats were housed and fed under the conditions previously described.

Data on control animals. For the experiment dealing with thymectomy, a

[1] This work was supported by a grant from the Penrose Fund of the American Philosophical Society and from the Rockefeller Foundation.
[2] Part of thesis studies submitted to the Faculty of the Graduate School of Medicine of the University of Pennsylvania in partial fulfilment of the requirements for the degree of Doctor of Science, D.Sc. (Med.).

special group of control rats designated as operative controls (animals subjected to the operative technic concerned in thymectomy without actual removal of the thymus gland) was used (3). A comparison of the growth and development of this control series with that of the control series of the Philadelphia Institute for Medical Research revealed a striking similarity in the data for both groups. For the sake of uniformity, therefore, these operative controls consisting of 208 animals, of which 183 or 87 per cent survived the 70-day period of observation, were used as controls in this experiment. The rate of growth as reflected by the average weight curve, and the data on somatic development are shown in figure 1 and table 1.

TABLE 1. COMPARISON OF SOMATIC DEVELOPMENT IN SUCCESSIVE GENERATIONS OF THYMUS IMPLANT GROUP WITH DEVELOPMENT OF CONTROLS

	Ears opened	Teeth erupted	Hair appeared	Eyes opened	Testes descended	Vagina opened
Controls	2–3 (2.9)[1]	7–10 (8.5)	11–14 (12.5)	13–17 (15.2)	24–35 (30.2)	39–50 (44.3)
F_1	2–4 (2.9)	7–9 (8.1)	12–16 (13)	13–16 (14.7)	25–35 (28.2)	39–56 (44.6)
F_2	2–3 (2.5)	6–9 (7.1)	11–13 (12.1)	9–16 (13.1)	19–30 (24.5)	37–52 (40.7)
F_3	2–3 (2.4)	4–7 (5.8)	8–12 (10.9)	7–14 (10.3)	18–25 (22.3)	35–43 (38.1)
F_4	2–3 (2.2)	3–8 (5.6)	8–13 (10.7)	8–15 (9.8)	18–22 (20)	32–39 (35.1)

[1] Figures in parentheses are average values (days).

RESULTS OF THYMUS IMPLANTATION IN SUCCESSIVE GENERATIONS OF RATS

Growth and development of test rats of the first generation (F_0). As a result of frequent homologous thymus implants in the original group of 6 normal animals, no effect on their growth and development was noted. The average interval between gestations was 37.8 days as compared with 28.3 days in the operative controls, and 37 days in the Philadelphia Institute for Medical Research series.

Second generation (F_1). A total of 84 rats was born in 10 litters to the original 3 pairs of normal animals receiving frequent homologous thymus implants. The litters varied in size from 6 to 10 animals, with an average of 8.4 animals per litter. Of the total of 84 rats, 76 or 90 per cent survived the 70-day period of observation.

The rate of growth and development of the second generation (F_1) thymus-injected test group as reported by Rowntree, Clark, Steinberg and Hanson (1), was but little affected in the animals of earlier litters born to the treated parents, but became accelerated in the later litters. In the thymus implant group, there was observed a slight increase in the rate of growth, as reflected in the average weight curve (fig. 1) and in the average weights at 10-day intervals (table 2). This increase in the rate of growth reached a maximum of 10 per cent at the age of 40 days.

No remarkable precocity was noted in the time of opening of the ears, eruption of the teeth, appearance of fur, opening of the eyes, descent of the testes, and opening of the vagina, although these somatic changes often occurred at or just before the early normal limit (table 1).

Third generation (F_2). A total of 113 rats was born in 15 litters of the third generation (F_2). The litters varied in size from 4 to 11, with an average of 7.5 animals per litter. Of the total of 113 rats, 101 or 89 per cent survived the 70-day period of observation.

TABLE 2. AVERAGE WEIGHT IN GRAMS AT 10-DAY INTERVALS OF SUCCESSIVE GENERATIONS OF THYMUS IMPLANT GROUP AND PERCENTAGE OVER THE WEIGHT OF CONTROLS

Age, days	Controls	F_1	% difference	F_2	% difference	F_3	% difference	F_4	% difference
Birth	4.9	5.0	2	5.1	4	5.1	4	5.2	6
10	15.0	15.5	3	17.4	16	17.6	17	18.0	20
20	28.5	29.4	3	33.0	16	34.0	19	34.9	23
30	49.1	51.9	6	55.6	13	57.2	16	58.1	18
40	68.6	75.6	10	77.8	13	79.8	16	81.2	18
50	93.5	97.3	4	97.9	5	100.3	7	100.8	8
60	113.3	118.2	4	118.9	5	119.6	5	122.0	8
70	135.3	138.9	3	142.0	5	142.8	5	142.3	5

The growth rate of the third generation (F_2) as reflected by the average weight curve was greater than either that of the controls or that of the second generation (fig. 1). This acceleration was greatest from the age of 10 to 40 days, reaching a maximum of 16 per cent at 10 to 20 days (table 2). In one exceptional litter, the test animals at the age of 12 days (fig. 2) were 73 per cent over the weight of their controls.

Table 1 summarizes the data relative to somatic differentiation. A number of instances of noteworthy precocity were observed. In 4 litters, the upper incisors erupted at 6 days, and in 6 litters at 7 days. In one litter, the eyes opened on the 9th day and in 5 litters on the 12th day. The computed average values for these changes were lower than those in the second generation (F_1), signifying mild but unquestionable precocity.

Fourth generation (F_3). A total of 157 rats was born in 20 litters of the fourth generation (F_3). The litters varied in size from 6 to 9, with an average of 7.9 animals per litter. Of the total of 157 rats, 134 or 85 per cent survived the 70-day period of observation.

The growth rate of the test animals of the fourth generation (F_3), as reflected by the average weight curve, was greater than that of the third generation (fig. 1), indicating a cumulation of effect in successive generations under treatment. This acceleration was greatest between the ages of 10 to 40 days, reaching a maximum of 19 per cent at the age of 20 days (table 2).

Striking evidences of precocious somatic development were noted. In one litter the upper incisors erupted on the 4th day, and in 7 litters on the 5th day. In 2 litters, the eyes opened on the 7th day, and in 3 litters on the 8th day.

Fifth generation (F_4). A total of 175 rats was born in 22 litters of the fifth generation (F_4). The litters varied in size from 6 to 11, with an average

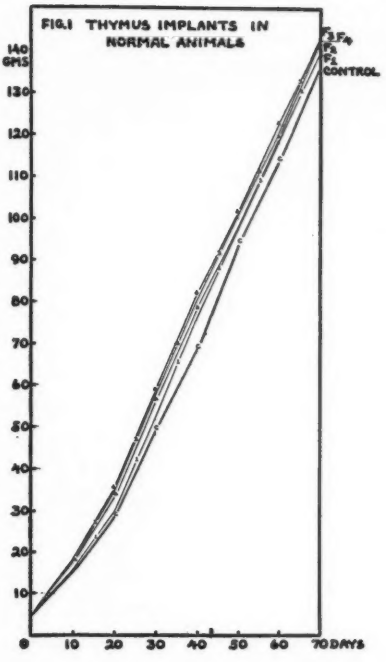

Fig. 1

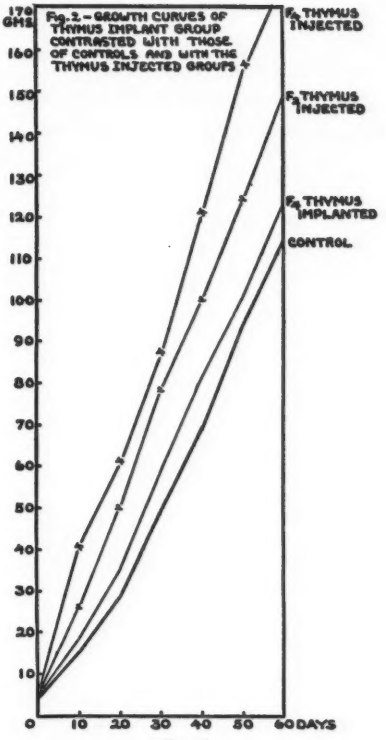

Fig. 2.

of 8 animals per litter. Of the total of 175 rats, 139 or 79 per cent reached the age of 70 days by February 1, 1937.

As in the preceding generations, the rats of the fifth generation (F_4) showed striking precocity in growth and development, as may be seen by reference to figure 1 and table 1. The most pronounced acceleration in growth occurred during the first 40 days of life, reaching a maximum of 23 per cent over the weight of the controls at the age of 20 days (table 2). The growth of the test rats of the fifth generation (F_4) as reflected by the average growth curve was greater than that of the fourth generation (F_3), again indicating an accumulation of effect in successive generations.

Precocity in somatic differentiation was as pronounced as was the growth of these animals. The upper incisors erupted on the 3rd day in one litter, on the 4th day in 2 litters, and on the 5th day in 7 litters. The test rats of the fifth generation (F_4) were robust, active, and in a number of instances were able, at the age of 10 to 12 days, to climb the walls of the cages and eat cracked corn. It was possible to wean all of the test rats of this generation by the end of the second week.

FATE OF IMPLANTS

It was noted that more of thymus implants sloughed in the normal animals

than in the thymectomized thymus implant animals. When implanted animals were sacrificed, areas of yellow pigmentation, about 1 cm. in diameter, were found subcutaneously in locations corresponding to the sites of implantations. These were sectioned and studied by Dr. J. H. Clark, pathologist to the Philadelphia General Hospital. He stated that the areas of pigmentation were made

TABLE 3. WEIGHTS AT 10-DAY INTERVALS OF FIFTH GENERATION (F_4) THYMUS IMPLANT GROUP CONTRASTED WITH THIRD (F_2) AND FIFTH GENERATION (F_4) THYMUS-INJECTED GROUPS, AND PERCENTAGE OVERWEIGHTS OF CONTROL ANIMALS

Age, days	Control	F_4 Thymus Implanted	% Over-weight	F_2 Thymus Injected	% Over-weight	F_4 Thymus Injected	% Over-weight
Birth	4.9	5.2	6	5.2	6	5.7	16
10	15.0	18.0	20	26.0	73	40.0	167
20	28.5	34.9	23	50.0	75	60.0	111
30	49.1	58.1	18	78.0	59	86.0	75
40	68.6	81.2	18	100.0	46	120.0	75
50	93.5	100.8	8	124.0	33	154.0	65
60	113.3	122.0	8	148.0	23	176.0	55

up of circumscribed areas of reticular tissue, resembling in every respect the tissue found in implanted thymectomized animals, and that this reticular tissue could be thymus in origin, but that unless he had known beforehand that these were thymus implants, he would not be justified in calling them thymus tissue. Low and high power photomicrographs of a thymus implant removed from a killed animal 6 weeks after implantation are shown in figures 4 and 5.

COMPARISON OF EFFECTS OF IMPLANTATIONS WITH THYMUS GLAND AND OF INJECTION OF THYMUS EXTRACT

In the 5 successive generations of rats under observation, homologous thymus implants made at weekly intervals resulted in a cumulative acceleration in growth and development. This precocity is comparable to, although not as great as, that produced by the injection of thymus extract into successive generations of rats (1). It may be seen by reference to table 3 and figure 2, that the rate of growth and development of the fifth generation (F_4) thymus implant strain is roughly equivalent to that of the third generation (F_2) injected strain, and considerably below the growth of the fifth generation (F_4) thymus extract injected strain, as reported by Rowntree, Clark, Steinberg and Hanson (1).

Reference to table 3 and figure 2 will show that, at the age of 30 days, an excess of 41 per cent is attained by the third generation (F_2) thymus-injected group over the fifth generation (F_4) thymus implanted strain. This difference diminished gradually although at 60 days, the end of the period of observation in the injected rats, it was still 15 per cent. In the fifth generation (F_4), thymus injected group, this acceleration in the rate of growth attained at the age of 10 days an excess of 147 per cent over the fifth generation (F_4) implanted strain, and at 60 days the injected rats still exceeded the implanted rats by 47 per cent. Similarly, the effect on somatic differentiation was not as striking in the implanted group as it was in the injected group. Reference to

table 4 will show that the lowest values recorded for opening of the ears, eruption of the teeth, appearance of hair, opening of the eyes, descent of the testes, and opening of the vagina correspond in a general way to the highest values noted in the thymus-injected group.

It would seem from this comparison that thymus implants (rat) provide a less effective stimulus to growth and development than does thymus extract (calf). These differences in biological effects obtained from thymus implants,

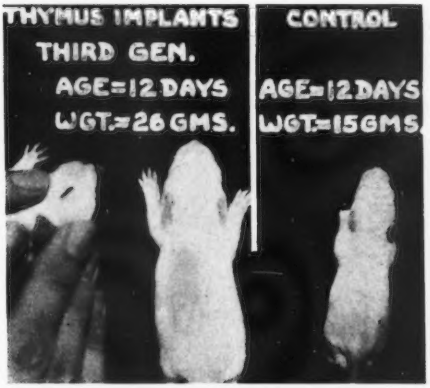

Fig. 3. Photograph of thymus implant rats (F_2) contrasted with control animals of same age.

as compared with thymus extract, may be due in part to the quantitative factor involved. As stated elsewhere, implantation began at the age of 30 days and, repeated until 12 implants had been made, was complete when the recipients were roughly 4 months of age. According to Donaldson (5), the maximum weight of the thymus gland in the rat is 0.291 gm. at the age of 48 days. Assuming that each implant weighed this amount, then the total weight of thymus tissue which each animal could possibly have received was 3.492 gm. During a similar period, the thymus-injected animals would have received not less than 90 daily injections of 1 cc. of thymus extract, which is equivalent to 90 gm. of thymus tissue. Actually, therefore, the injected animal received the equivalent of over 25 times as much thymus tissue as the implanted animal.

DISCUSSION

This investigation has been carried on for more than 2 years, and is based on observations of 743 animals in 5 generations distributed as follows: operative controls 208, first generation (F_0) 6, second generation (F_1) 84, third generation (F_2) 114, fourth generation (F_3) 157, and fifth generation (F_4) 175.

In a previous communication, it was demonstrated that as a result of thymectomy in successive generations, the offspring are retarded in growth, this retardation reaching a maximum of 14 per cent at the age of 30 days in the test rats of the fifth generation (F_4) (3, 4). By means of 12 homologous implants

made in the manner described to successive generations of thymectomized parents, the rats of the fifth generation (F_4) not only failed to show this retardation, but they showed a stimulation to growth comparable to that which obtains from implants in normal rats. Actually, therefore, implants have pro-

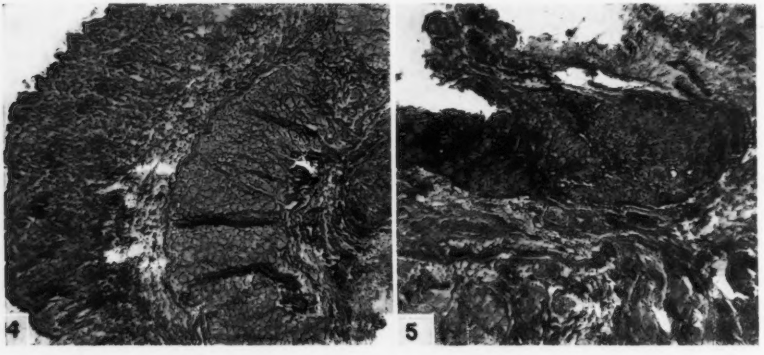

Fig. 4. Low power photomicrograph of a thymus implant removed from a rat killed 6 weeks after implant was made.
Fig. 5. High power photomicrograph of a thymus implant removed from a rat killed 6 weeks after implant was made.

vided a better stimulus to growth in the thymectomized than in the normal animal.

In addition to these biological effects, there were noted other manifestations which have not been so well emphasized. Thus fifth generation (F_4) test animals in a number of instances were, at the age of 10 to 12 days, able to climb the walls of the cages, and to eat cracked corn. Weaning was possible at the age of 14 days, and the young had no need of further care from the parents. These animals appeared to be in good health, and their reactions, asleep or awake, resembled in all respects those of normal rats 24 to 28 days of age.

Implantation began at the age of 30 days and, repeated at weekly intervals until 12 had been made, was completed when the animals were roughly 4 months old. The average ages at which the first pregnancy, and the casting of the first litter occurred, were 70 and 91 days respectively. By 120 days, or just after completion of the entire series of 12 implants, most parent rats were either pregnant the second time, or had cast a second litter. Thus the first litter was usually cast after the 8th or 9th implant, and the second litter shortly after the 12th implant. As a rule, the rate of growth and development showed the greatest acceleration in the second litter, although frequently the first and second litters were about equal. The third litter, born some weeks subsequent to the last implant, often showed evidences of failing precocity, and the fourth and fifth litters, born months later, showed little or none at all. In one instance, a female was permitted to cast 6 litters. The first two born during the period of implantation showed striking precocity, while the remaining litters born subsequently showed no precocity at all. This would seem to indicate that the stimulus to growth and development provided by the 12 implants was ex-

hausted shortly after the last implant. In another instance, a female rat of the third generation (F_2) cast her first litter at the age of 114 days, or 2 days after the last of a series of 12 implants. This litter showed striking precocity in growth and development. The second litter, cast at the age of 147 days, showed practically no precocity. A second series of implants was given at weekly intervals, until 6 additional implants had been given. The third litter was cast at

TABLE 4. COMPARISON OF SOMATIC DEVELOPMENT IN THYMUS IMPLANT AND THYMUS-INJECTED GROUPS

	Ears opened	Upper incisors erupted	Hair appeared	Eyes opened	Testes descended	Vagina opened
Control	2–3 (2.9)[1]	7–10 (8.5)	11–14 (12.5)	13–17 (15.2)	24–35 (30.2)	39–50 (44.3)
F_4 Thymus Implanted strain	2–3 (2.2)	3–8 (5.6)	8–13 (10.7)	8–15 (9.8)	18–22 (20)	32–39 (35.1)
F_2 Thymus Injected strain	2	4–6	4–6	4–6	15–21	23–32
F_4 Thymus Injected strain	1–2	2–3	2–3	2–3	6–10	18–20

[1] Figures in parentheses are averages (days).

the age of 190 days so that the parent female had received 3 implants before her third pregnancy, and 3 implants during this pregnancy. This third litter also showed no precocity. It would seem therefore that the stimulation to growth and development by thymus implants depends upon continued treatment, and that this stimulus when lost is difficult to reestablish.

It is realized that the designation of the exact time of opening of the ears, the appearance of fur, and the descent of the testes depends, to some extent, upon the ability and experience of the person who makes the observations. Of all the vital phenomena concerned in somatic differentiation, the eruption of the upper incisors and the opening of the eyes are probably the two most clearly defined changes noted in the development of the rat, and the personal equation is but little, if at all, concerned in the recognition of these phenomena. In this presentation, emphasis has therefore been placed on these two somatic changes.

SUMMARY

From observations based on a study of 535 test animals and 208 controls, it appears that frequent homologous thymus implants in normal parent rats have resulted in an accruing acceleration in the rate of growth and development in 4 generations of offspring. This precocity is comparable, although not as marked as that resulting from the continuous administration of 1 cc. of thymus extract (Hanson) daily in successive generations.

The stimulus to growth and development provided by frequent thymus implants is of short duration, lasting only so long as additional implants are made and disappearing rapidly after implantation is discontinued.

REFERENCES

1. Rowntree, L. G., J. H. Clark and A. M. Hanson: J.A.M.A. **103**:1425. 1934, *ibid.*, Tr. A. Am. Physicians **49**:252. 1934, *ibid.*, Arch Int. Med. **56**:1. 1935.
Rowntree, L. G., J. H. Clark, A. Steinberg and N. H. Einhorn: Tr. A. Am. Physicians **51**:148. 1936.
2. Einhorn, N. H., and L. G. Rowntree: Science **84**:2166. 1936.
3. Einhorn, N. H., and L. G. Rowntree: Endocrinology **20**:342. 1936.
4. Einhorn, N. H., and L. G. Rowntree: Science **83**:443. 1936.
5. Donaldson, H. H.: The Rat, Ed. 2, Memoirs of the Wistar Institute of Anatomy and Biology No. 6, Philadelphia, 1924.

INFLUENCE OF ADRENALECTOMY UPON THE SEX DIFFERENCE IN KETOSIS

EATON M. MAC KAY AND RICHARD H. BARNES
From the Scripps Metabolic Clinic
LA JOLLA, CALIFORNIA

The higher level of ketonuria during fasting in women than in men was first noted by Deuel and Gulick (1). Butts and Deuel (2) have found that an artificial ketonuria, produced by administration of sodium acetoacetate to rats, also showed similar sex differences. The discovery by Burn and Ling (3, 5) and others (4) that preparations of the anterior pituitary gland have the ability to produce a ketonuria in fasting rats that otherwise have no measurable quantity of ketone bodies led Butts et al (6) to consider the possibility that the sex difference in ketosis rested in the activity of the pituitary gland. It has been found (7, 8, 9) that the ketosis due to APE is greatly reduced or abolished following the removal of the adrenal glands. Furthermore, the fasting ketosis of pregnancy (9), which might well be related to anterior pituitary activity, is abolished by adrenalectomy. It would seem possible that if the sex difference in ketosis was a function of the anterior pituitary it might disappear when the adrenal glands are removed.

The ketolytic activity of the female is less than that of the male, so that when acetoacetate is fed in similar quantities the male rats excrete less (2) than females. We have found that this is true for the sodium salt of racemic β-hydroxybutyric acid as well (10). A perusal of the data (10) on ketonuria after adrenalectomy suggests that this procedure minimizes the sex difference, and a summary of these experiments (table 2) shows very clearly that this is the case.

In order to examine the influence of adrenalectomy on the sex difference in ketosis more fully, experiments were carried out in which the ketosis was not produced by the administration of preformed ketone bodies. Higher fatty acids (11) such as butyric and caprylic were fed in the form of their sodium salts. When a diet high in fat and low in protein (and probably choline) is fed for two weeks or more, the liver becomes very fatty (12). Fasting of these rats results in a very heavy ketonuria, which is greater in female rats than in male rats (tables 1 and 2). In experiment 3 the caprylate was fed as a 7.5 per cent solution in a dose of 150 mg. the first day, and after that a 5 per cent solution in a dose of 100 mg. per 100 sq. cm. body surface per day. In experiment 4, over the 2.5 days that the urine specimen covered, 214 mg. of sodium butyrate per U of body surface was fed in 4.28 per cent solution. The rats in experiment 5 had been on the high fat, low protein diet (12) for 16 days before fasting, and urine collections were commenced. The rats were given 3 cc. of

TABLE 1

Exp. no.	Group	No. rats	Sex	Body wt. gm.	Body surface sq. cm.	Urine excretion in mg. per 100 sq. cm. body surface per day								
						Ketone bodies days					Nitrogen days			
						1	2	3	4	5	1	2	3	4
Sodium caprylate fed														
3	C	4	♂	284	490	8	15	34	22		33	26	25	23
	Ad	4	♂	285	491	7	11	18	11		24	17	11	8
	C	4	♀	201	389	26	31				76	51		
	Ad	4	♀	201	389	6	12				44	29		
Sodium butyrate fed														
4	C	3	♂	213	405	6					27			
	Ad	3	♂	210	402	5					18			
	C	3	♀	155	327	9					28			
	Ad	3	♀	159	333	4					16			
Fasting after a diet producing a high liver fat														
5	C	3	♂	220	413	0	9	17	24	11				
	C	3	♂	223	417	1	19	13	21	13				
	Ad	3	♂	227	422	0	1	2	1	0				
	Ad	3	♂	226	421	1	4	3	1	0				
	C	3	♀	208	399	0	11	20	26	33				
	C	3	♀	211	403	1	19	25	40	24				
	Ad	3	♀	210	402	1	2	4	2	0				
	Ad	3	♀	208	399	1	2	4	1	0				

TABLE 2. INFLUENCE OF ADRENALECTOMY UPON THE EFFECT OF SEX ON KETOSIS. AVERAGE EXCRETION OF KETONE BODIES AS MG. ACETONE PER 100 SQ. CM. BODY SURFACE PER DAY

Exp.	No. Rats per Grp.	No. Obs. per Grp.	Males		Females	
			Controls	Adrenalectomized	Controls	Adrenalectomized
Sodium aceto-acetate fed						
1[1]	12	40	23	10	35	11
Sodium B-hydroxybutyrate fed						
2[1]	7	14	24	19	28	20
Sodium caprylate						
3	4	8	12	9	28	9
Sodium butyrate						
4	3	1	6	5	9	4
Fasting after a diet producing a high liver fat						
5	6	10	13	1	20	2

[1] The data for these experiments have been presented in detail elsewhere (10). The other experiments are given in table 1.

1 per cent sodium chloride solution by stomach tube each day, in order to insure good urine volumes. Our method of removing the adrenals (13) and reasons for commencing observations immediately after the operation have been described elsewhere (10).

When the average degrees of ketosis (as ketonuria) are compared for all those experiments with comparable groups (table 2), it can be seen that the sex difference is practically abolished in the absence of the adrenal glands. The reduction in the sex difference in ketosis caused by adrenalectomy is particularly interesting, in view of the larger volume of adrenal cortex tissue which the female rat possesses (13), for this is the adrenal tissue which appears to be concerned in ketosis (8, 14).

SUMMARY

Following adrenalectomy, the sex difference in ketosis, as measured by the ketonuria following the administration of ketone bodies, higher fatty acids, or fasting with a dietetically produced fatty liver, tends to disappear. The probable explanation of this action is that the sex difference in ketosis is dependent on anterior pituitary activity, and the ketogenic factor of this gland does not act in the absence of the adrenal cortex.

REFERENCES

1. Deuel, H. J., Jr., and M. Gulick: J. Biol. Chem. **96**:25. 1932.
2. Butts, J. S., and H. J. Deuel, Jr.: J. Biol. Chem. **100**:415. 1933.
3. Burn, J. H., and H. W. Ling: J. Physiol. **69**:xix. 1930.
4. Anselimino, K. J., and F. Hoffman: Klin. Wchnschr. **10**:2380. 1931.
5. Burn, J. H., and H. W. Ling: Quart. J. Pharm. & Pharmacol. **2**:1. 1933.
6. Butts, J. S., C. H. Cutler and H. J. Deuel, Jr.: J. Biol. Chem. **105**:45. 1934.
7. Fry, E. J.: Am. J. Physiol. **116**:55. 1936.
8. MacKay, E. M., and R. H. Barnes: Am. J. Physiol. **118**:184. 1937.
9. MacKay, E. M., and R. H. Barnes: Proc. Soc. Exper. Biol. & Med. **34**: 682. 1936.
10. MacKay, E. M. and R. H. Barnes: Am. J. Physiol. In press.
11. Butts, J. S., C. H. Cutler, L. Hallman and H. J. Deuel, Jr.: J. Biol. Chem. **109**:597. 1935.
12. Channon, H. J., and H. Wilkinson: Biochem. J. **29**:350. 1935.
13. MacKay, E. M., and L. L. MacKay: J. Exper. Med. **43**:395. 1926.
14. Evans, G.: Am. J. Physiol. **114**:297. 1936.

CRITERIA FOR THE SELECTION OF ESTROUS RABBITS

THE SIGNIFICANCE OF SEASONAL FACTORS[1]

MAURICE H. FRIEDMAN
From the Department of Physiology, University of Pennsylvania Medical School
PHILADELPHIA, PENNSYLVANIA

Most of the investigators who have had occasion to use estrous rabbits have selected animals which have been isolated for 30 days (or more), or which present a large, purple vagina upon examination. It is implied that all such rabbits are actually in estrus. However, no investigator who has used such criteria has ever presented any data to establish their reliability. On the contrary, there is evidence by Hammond and Marshall (1) that estrus does not occur with regularity in isolated animals, and that the vaginal color is no guide as to the presence of heat. Over a period of several years the experience of this laboratory has fully confirmed Hammond and Marshall in every particular, despite the fact that the two colonies of animals are maintained under very different conditions. Nevertheless, there appeared to be little justification for the publication of our data until the use of these unsatisfactory criteria became so general. This report is now presented to emphasize the errors in the current practice, to indicate how estrous rabbits may be selected with much greater consistency, and to call attention to the importance of seasonal influences on the mating behavior of the laboratory rabbit.

MATERIAL AND METHODS

Animals. The rabbits were purchased from a single source, where they were kept in individual, outdoor cages, strictly isolated from other females as well as from males. The cages all faced south and were protected from draughts. The feed at the rabbitry consisted of a dry grain mixture supplemented by alfalfa hay and water.[2] The rabbits were shipped to the laboratory in individual boxes and were in transit no more than 20 hours.

All breeds were represented, so that the weights of the mature animals varied from 1.8 kg. (Polish) to 5.3 kg. (giant chinchilla and New Zealand). Over half the animals were of the chinchilla variety, and inasmuch as the results with the latter did not differ from the results obtained with the other varieties the data are not classified as to breed.

Laboratory care and handling. Upon arrival at the laboratory, the animals

[1] This work was supported by a grant from the Rockefeller Foundation.

[2] From personal inspection the author can testify as to the excellence of the conditions under which the stock was maintained, and the adequacy of the provision for strict isolation. It is noteworthy that, among the 160 animals purchased as isolated females, not a single pregnancy was found except those resulting from the matings observed in the laboratory. Moreover, the high fertility rate of these matings would have been impossible if a significant number of the females had been pseudopregnant at the time of the first laboratory mating.

were permanently numbered by an ear tatoo and carefully inspected for snuffles, ear tick, diarrhea, and any other grossly detectable abnormality. Any animals found to be abnormal or suspected of snuffles were quarantined. Only completely healthy animals were introduced into the colony, where individual cages isolated each animal from all others. The diet was constant throughout the period of observation. It consisted of 200 gm. of feed[3] wetted sufficiently to form a firm paste. The food consumption of each animal was recorded daily. When on a few occasions mild epidemics of snuffles broke out in the colony, all stricken animals were removed from the stock.

The detection of estrus and the breeding records. The presence of estrus was determined by the introduction of the female into the cage of the male. If the doe accepted service, she was judged to be in heat. If she persistently refused one or more vigorous bucks, she was listed as anestrous. In a very

TABLE 1. THE INCIDENCE OF ESTRUS IN ISOLATED RABBITS

	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	July	Yearly Total
First Matings[1]	24	22	20	8	8	16	17	21	5	15	3	160
Copulated	8	6	5	5	5	11	14	18	3	12	0	88
Estrus, %	33	27	25	—	—	68	82	86	—	80	—	55
Total Matings[2]	32	41	37	42	14	20	24	23	10	19	7	269
Copulated	15	9	10	16	9	13	17	19	4	13	1	125
Estrus, %	47	22	27	38	64	65	71	83	40[3]	69	—	45

[1] Listed in this line are the numbers of animals mated for the first time after their arrival at the laboratory.
[2] Under the heading total matings are listed all trials of isolated animals, including animals which had refused service at previous matings.
[3] The laboratory temperature in May was uncomfortably high, due to the appearance of warm weather at a time when the steam had not been turned off. The passage of the steam mains close to the animal quarters made adequate temperature control impossible at such times.

few instances where there was any doubt as to successful penetration the results were checked by an examination of the vaginal smear for sperm. The records submitted cover a period of 2 years and include observations in every month except August. They consist exclusively of the records of disease-free animals consuming 75 per cent or more of their daily ration. Special importance is attached to the records of food consumption, because anorexia so regularly preceded the appearance of more gross signs of illness. This is particularly true in the last third of pregnancy. The appearance of anorexia at such time usually heralded the onset of an illness lacking in other symptoms until it prostrated the animal at or near term, or ended in a protracted and abnormal labor. No female suffering such abnormal labor was included in the group of postpartum females.

PROCEDURES AND RESULTS

Isolated females. One hundred and sixty animals were used for these observations. Among them were some virgin females 8 to 10 months old,

[3] Whole ground wheat, 57; alfalfa leaf meal, 40; soy bean oil meal, 3 parts.

which had been isolated since the age of 4 months at the rabbit farm. Most of the females, however, were somewhat more mature, and some of them had borne one litter 2 or more months previously. About two-thirds of these isolated females were tried with a male 7 to 10 days after their arrival in the laboratory. The rest of the animals were tested only after they had been in the laboratory 3 weeks or longer. No difference in the success of the matings was noted, whether the females had been in the laboratory for a short period or for 3 weeks or longer, at the time of the first observation. The results of these initial matings are given in the upper part of table 1.

The 72 females which refused service at the initial mating were tried again from 7 to 30 days later and, if necessary, again at similar intervals until service was accepted, or until the female was shown to be persistently anestrous. In this manner, the 72 animals which were not in heat at the initial mating were observed at 109 subsequent trials. Consequently, a total of 269 observations were made on the 160 isolated rabbits (lower portion of table 1).

TABLE 2. A COMPARISON OF THE BREEDING PERFORMANCE OF THE VARIOUS GROUPS OF RABBITS AT THE INITIAL MATINGS

Group	Number of Animals	Copulated	Estrus %
Isolated	160	88	55
Postpartum	71	66	93
Assay (postpartum)	99	94	95
Post-pseudopregnant	104	96	92

Postpartum, post-pseudopregnant, and assay females. The postpartum females were mated 2 to 30 days after casting a litter, the young having been removed within 24 hr. postpartum. The assay animals were choice postpartum females in which laparotomy had disclosed that the injection of a small dose of gonadotropic extract (0.5 minimal effective dose or less) had produced no detectable ovarian change. These females were mated 4 to 16 days postoperatively and 8 to 32 days postpartum. The rabbits listed as post-pseudopregnant had failed to litter after an earlier mating, or had ovulated in response to the injection of a gonadotropic extract. These animals were mated 20 to 50 days after the infertile coitus or injection (1 to 30 days after the end of the period of pseudopregnancy). In so far as some of these animals may have failed to ovulate after the infertile coitus, this listing is acknowledged to be one of convenience rather than one of strictest accuracy. At any rate, the performance of the several types of females may be compared in table 2. Here only the initial matings are listed. The subsequent matings of the few anestrous postpartum and post-pseudopregnant females are not included.

The relation of vaginal color to the occurrence of estrus. The vaginal colors indicated in table 3 were noted immediately preceding the introduction of the female into the cage of the male. Listed in this table are the observations at the time of 217 of the 269 matings of the isolated animals, and at the time of 233 of the 274 *initial* matings of the other animals. Vaginal examination was not made at the time of the rest of the matings. Tabulation of only

the initial matings of the isolated females would not alter the figures except to reduce the number of observations. Of course, the readings of the colors are not entirely objective, but, on the basis of readings made by visitors to the laboratory and by colleagues, it is assured that the two extremes of the scale are definite enough to be beyond question. The exact classification of the intermediate colors was not always agreed upon, the most frequent discrepancies appearing in the listing of the presence and degree of the blue tint which is, or resembles, cyanosis. It is barely possible, although improbable, that some observer might classify as purple the vulva with 2+ of the blue tint. None of the visitors or colleagues, however, ever confused such vulva with the frankly purple vagina referred to by most breeders.

DISCUSSION

The data are in excellent agreement with the data of Hammond and Marshall in every respect. To be sure, the incidence of estrus in the isolated

TABLE 3. THE RELATION OF VAGINAL COLOR TO MATING BEHAVIOR

	Isolated Females			All other Females		
Color[1]	Number	Copulated	Estrus %	Number	Copulated	Estrus %
Pale	42	11	26	19	15	79
Pink	46	22	48	55	51	93
Pink,+	33	15	47	48	42	87
Pink,++	19	7	37	25	24	96
Red	28	20	70	32	32	100
Purple	49	32	65	54	51	94

[1] Pale = Practically colorless mucous membrane.
Pink = Diffusely colored with no trace of blue; resembles normal human nail bed when hand is held above the heart.
Pink + = pink background with very slight tinge of blue coloration as seen frequently in the normal human nail bed when arm hangs at rest.
Pink + + = With more definite blue tinge; general impression still pink.
Red = diffusely and more intensely colored; with little or no trace of blue; color of normal human lip or deeper.
Purple = Frankly engorged and purple; usually very large vulva.

does of the Cambridge colony shows an all-year average of 63 per cent contrasted with 55 per cent of the initial matings and 45 per cent of all matings of isolated females in the Philadelphia colony. This difference between the two sets of data is easily understood, when one considers that only 35 per cent of the matings in the Cambridge colony were made in the autumn season (September to December inclusive). In the data of the present report, the autumn matings account for 46 per cent of the initial matings and 57 per cent of the total matings. Actually the monthly variations in the incidence of estrus in isolated animals are almost identical in the two colonies, varying from 25 per cent in October and November to approximately 80 per cent in April. In view of the fact that the Cambridge colony is housed outdoors and is not fed a uniform diet the year around, this agreement is striking. The constancy of the diet and the minor variations in room temperature in the Philadelphia colony strongly suggest some other environmental influence, possibly light, as the cause of these seasonal trends.

Because of the error involved in the use of isolated females, a trustworthy

sign of estrus would be of considerable value. Some authors have assumed that the criterion of vaginal color is of particular value in the chinchilla rabbit. It will be recalled that over one-half the animals considered in this report were chinchillas. It should be emphasized that the correlation of vaginal color and estrus was no better in this breed than in the other breeds. Table 3 presents the criterion of vaginal color in the best possible light, and from this presentation one might expect that about two-thirds of isolated rabbits with purple vaginae would be in heat. Yet if this criterion were employed during the autumn, when some dependable sign is most needed, the error would be considerably greater. From September to December inclusive, purple vulva were observed 27 times in isolated females. In only 11 instances were the does in heat, an incidence of 40 per cent compared with 33 per cent in all isolated does, irrespective of vaginal color, over the same period of time. That there is no essential physiological relation between vaginal color and estrus is seen from the fact that only a small fraction of isolated estrus animals display a purple vagina, and the same is true in the postpartum and post-pseudopregnant groups.

The excellent performance of the select group of animals (post-partum, post-pseudopregnant and assay animals) affords thorough confirmation of the data of Hammond and Marshall. These authors state that "no cases have been met with where does in the breeding season refused to copulate within a few days of parturition if the young are removed at birth." No exact definition of the breeding season was given, but it certainly does not include October and November. Until the past 2 years there had appeared no instance in which a postpartum female of our colony had refused service if mated from 2 to 30 days postpartum. The records indicate, however, that up to the winter of 1935 no matings of such animals had been attempted before the middle of December. Consequently, the significance of the seasonal influence was not appreciated until the records of the present series were examined. This examination discloses 5 cases of anestrum in postpartum (unoperated) females. All 5 were encountered in October and November, during which time 36 of the 71 matings in this group were conducted. Among the laparotomized postpartum females (assay group) 3 of the 5 cases of anestrus occurred in these same months. The 2 remaining cases in this group were in animals mated 4 days postoperatively. Both animals accepted service a few days later. Similarly, 6 of the 8 cases of anestrus in the post-pseudopregnant group were seen in October and November. It is therefore difficult to avoid consideration of the seasonal influences, even in the mating of these select groups.

Finally, consideration must be given to the method employed for the detection of estrus, since pregnant and pseudopregnant rabbits will frequently copulate, although unable to ovulate. Such instances of heat behavior are admittedly not comparable to the manifestations of heat in other mammals, in which coitus is permitted only by non-pregnant females at a restricted time interval just preceding ovulation. If the term 'estrus' is applied to the rabbit at all, it would seem expedient to define it as a condition in the female characterized by manifestations of heat behavior at a time when the probability of ovula-

tion following coitus approximates that found in other mammals. In the present series of breeding experiments it is impossible to state exactly just what percentage of copulations were followed by ovulation, since routine laparotomies were not performed. From the number of litters borne, however, it is evident that ovulation followed coitus in at least 72 per cent of the isolated animals and at least 80 per cent of the postpartum group. This compares satisfactorily with the 84 per cent fertility in matings of carefully selected estrus rats (2). Consequently, coitus should be an acceptable indication of estrus in a rabbit whose physiological condition does not preclude the possibility of ovulation.

As examined by this indication of estrus, the criteria by which estrous rabbits are currently selected are obviously untrustworthy. It is equally obvious that estrous rabbits can be selected with a very high degree of consistency if the selection is limited to postpartum or post-pseudopregnant animals, particularly if due consideration is given to the influence of seasonal factors.

SUMMARY

There is a marked seasonal variation in the incidence of estrus in the laboratory rabbit. It is suggested that some factor other than diet or temperature is responsible. There is no justification for the selection of estrus rabbits,, either on the basis of the duration of isolation, or on the basis of vaginal color. Estrous rabbits can be obtained with consistency if the choice is limited to healthy postpartum females. Ths is particularly true if proper consideration is given to the seasonal influence.

REFERENCES

1. Hammond J., and F. H. A. Marshall: Reproduction in the Rabbit, Oliver & Boyd, London, 1925.
2. Evans, H. M., and K. S. Bishop: J. Metab. Research 3:210. 1923.

THE EFFECT OF SYNTHETIC ANDROGEN UPON THE GONADOTROPIC POTENCY OF THE ANTERIOR PITUITARY

JAMES B. HAMILTON AND J. M. WOLFE
From the Departments of Anatomy, Yale University School of Medicine
NEW HAVEN, CONNECTICUT
and Albany Medical College, Union University
ALBANY, NEW YORK

Since the original discovery (1, 2) that implantation of anterior lobe tissue stimulated precocious sexual maturity in immature rodents, this method has been used to test the gonadotropic potency of the pituitaries of animals in various conditions (3, 4, 5). By utilization of this technic, the following data has been obtained concerning the influence of the injection of a synthetic androgenic substance, testosterone propionate, upon the gonadotropic potency of the anterior pituitaries of normal and castrated male and female rats. Moore and Price (6) have previously reported that injection of androsterone decreased the stimulating potencies of the pituitaries of young male rats.

METHODS AND MATERIALS

In our studies, pituitaries from androgen-injected donor rats were implanted on successive days in 21 to 23-day-old female rats; the anterior lobe tissue was introduced into the thigh musculature of the test rats by means of a trocar. The immature test animals or hosts were sacrificed 100 hr. after the first implantation. The ovaries and uteri were weighed, and observations made with regard to the opening of the vagina.

Control observations were made by implanting into litter-mate hosts pituitaries from control donors which had been subjected to the same conditions as experimental donors, save for the absence of androgen injections. In some litters one immature rat, which received no implantation, was sacrificed for comparison with littermate hosts which had received pituitaries from androgen-injected and control donors. In these experiments, 82 adult donors were used; their pituitaries were implanted into 49 hosts, taken from 14 litters. The experimental donors received 500 gamma of testosterone propionate daily for periods of time indicated in table 1. Since the amount and type of the oil vehicle influences the rate of absorption (7), one-tenth of 1 cc. of peanut oil was used as a vehicle for each injection.

RESULTS AND DISCUSSION

Data from the immature test animals, presented in table 1, showed uniformly that, in hosts which had received pituitary implants from donors injected with testosterone propionate, there was less growth of the ovaries

TABLE 1. AS TESTED BY OVARIAN AND UTERINE WEIGHT FOLLOWING PITUITARY IMPLANTATION INTO IMMATURE FEMALE RATS, PITUITARY GONADOTROPIC PROPERTY IS GREATLY REDUCED BY ADMINISTRATION OF TESTOSTERONE PROPIONATE

No. Litter	No. Animal	Das. of Age at autopsy	Bdy. Wt. in gm.	Ovar. Wt. in mg.	Uter. Wt. in mg.	Vagina	Bdy. Wt. in gm.	Sex	Condition	Period of Injection in Days
			Hosts						*Donors*	
K	35	27		20	20	Closed	200–240	M M	Normal Normal	30 30
	36	27		40	82	Open	200–240	M M	30-day Normal 30-day Normal	Control Control
	37	27		16	17	Closed	200–240		No implants	
J	32	26		16	24	Closed	200–240	M M	Normal Normal	30 30
	33	26		20	130	Open	200–240	M M	Normal Normal	Control Control
	34	26		16	16	Closed	200–240		No implants	
N	45	27	55	Small	Small	Closed	230 206	M M	Normal Normal	15 15
	46	27	51	Small	Small	Closed	260 200	M M	Normal Normal	15 15
	47	27	49	Large	Large	Open	196 176	M M	Normal Normal	Control Control
I	30	25	39	14	86	Closed	185 205	M M	Normal Normal	30 30
	31	25	38	9	42	Closed			No Implants	
H	27	26	45		24	Closed	201 190	F F	Normal Normal	30 30
	28	26	43		27	Closed	204 196	F F	Normal Normal	30 30
	29	26	46		58	Open	209 198	F F	Normal Normal	Control Control
E	13	25	38	24	90	Open	298 329	M M	Castrate Castrate	13 14
	14	25	43	20	48	Open	202 272	M M	Castrate Castrate	13 14
	15	25	42	28	136 fluid	Open	216 256	M M	13-day Castrate 14-day Castrate	Control Control
	16	25	41	21	58	Open	240 197	M M	Castrate Castrate	14 15
	17	25	34	9	27	Closed			No implants	
F	18	27	45	25	107	Open	283 189	M M	14-day Castrate 15-day Castrate	Control Control
	19	27	50	22	45	Closed	284 230	M M	Castrate Castrate	13 14
	20	27	52	17	45	Open	201 266	M M	Castrate Castrate	13 15
	21	27	52	23	46	Open	150 196	M M	Castrate Castrate	14 15
	22	27	53	13	19	Closed			No implants	
D	10	27	49	24	223	Open slightly	163 251 144	M M M	Castrate Castrate Castrate	5 6 7
	11	27	47	32	392	Open	191 179 148	M M M	5-day Castrate 6-day Castrate 7-day Castrate	Control Control Control
	12	27	44	10	53	Closed			No implants	
M	41	28		28	Small	Open	200–240	M M	Castrate Castrate	30 30
	42	28		26	Small	Closed	200–240	M	Castrate	30

TABLE 1 (*Continued*)

No. Litter	No. Animal	Das. of Age at autopsy	Bdy. Wt. in gm.	Ovar. Wt. in mg.	Uter. Wt. in mg.	Vagina	Bdy. Wt. in gm.	Sex	Condition	Period of Injection in Days
			Hosts						*Donors*	
M								M	Castrate	30
	43	28		106	Large	Open	200–240	M M	30-day Castrate 30-day Castrate	Control Control
	44	28		168	Large	Open	200–240	M M	30-day Castrate 30-day Castrate	Control Control
L	38	28		36	Small	Open slightly	200–240	M M	Castrate Castrate	30 30
	39	28		58	Large	Open	200–240	M M	30-day Castrate 30-day Castrate	Control Control
	40	28		173	Large	Open	200–240	M M	30-day Castrate 30-day Castrate	Control Control
A	1	24	63	10	42	Open slightly	224 222	M M	10-days Castrate before injections begun	10 10
	2	24	65	22	53	Open slightly	172 182	M M	10-days Castrate before injections begun	10 10
	3	24	60	38	233 much fluid	Open wide	171 197	M M	20-days Castrate	Control Control
	4	24	67	50	64	Open wide	216 198	M M	20-days Castrate	Control Control
	4a	24	68	14	25	Closed			No implants	
B	5	25	64	17	44	Open slightly	134 165	M M	10-days Castrate before injections begun	10 10
	6	25	62	36	124	Open wide	136 180	M M	20-days Castrate	Control Control
	6a	25	63	11	22	Closed			No implants	
C	7	24	35	19	41	Closed	184 146 190	M M M	10-days Castrate before injections begun	10 10 10
	8	24	36	31	116	Open	140 160 144	M M M	20-days Castrate 20-days Castrate 20-days Castrate	Control Control Control
	9	24	31	12	16	Closed			No implants	
G	23	27	49	39	90	Open	304 164	M M	13-days Castrate before injections begun	5 6
	24	27	44	55	77	Open	308 176	M M	18-days Castrate 19-days Castrate	Control Control
	25	27	40	80	121	Open	220 185	F F	21-days Castrate 22-days Castrate	Control Control
	26	27	45	26	35	Closed	200 202	F F	Castrate Castrate	21 22

and accessory reproductive organs than in those animals whose implants had come from uninjected donors. The average ovarian and uterine weights of hosts receiving implants from injected donors was 23.3 mg. and 77.3 mg. respectively (table 2). In hosts receiving pituitaries from uninjected donors, the mean ovarian weight was 65 mg., the mean uterine weight 184.5 mg. Due to the variability in the amount of retained fluid, however, the uterine weights were not considered as accurate an index of the ovarian activity as the weights of the ovaries themselves. Opening of the vagina occurred in all hosts re-

ceiving implants from non-injected donors, but in only 73 per cent of hosts receiving implants from injected castrates, and never in hosts whose implants were from injected noncastrates.

Pituitaries from castrated rats exhibit greater ovary-stimulating properties than do those of normal rats, particularly when a long post-castration time elapses before implantation (2, 3). Thus, the greatest difference in gonadotropic properties of the anterior pituitaries of injected and control animals occurred in those which were castrated for long periods, and in which the experimental donors had received injections throughout this period (see litters *L* and *M*, table 1). If the limited number of animals used in the present investigation provides an accurate index, the amounts of testosterone propionate used in these experiments failed to keep the pituitary gonadotropic

TABLE 2. AS TESTED BY OVARIAN AND UTERINE GROWTH IN IMMATURE LITTER-MATE HOSTS, ANDROGEN INJECTIONS LESSENED BUT DID NOT ELIMINATE (A) THE GONADOTROPIC PROPERTIES OF THE PITUITARY IN NON-CASTRATED RATS AND (B) THE INCREASED GONADOTROPIC CAPACITY IN CASTRATED RATS

Donors	Hosts			
Donors From Which Implants Were Received	No. of Rats	Average Ovarian Wt. (mg.)	Average Uterine Wt. (mg.)	% Showing Opening of Vagina
Androgen-injected (Castrated and non-castrated)	23	23.3	77.3	60
Control, non-injected (Castrated and non-castrated)	17	65.0	184.5	100
No implants	9	12.7	23.0	0
Androgen-injected (Non-castrated)	7	16.6	40.2	0
Androgen-injected (Castrated)	15	22.8	53.0	73.3

capacity of injected castrated rats down to the level achieved in non-castrated injected rats. Hosts receiving implants from non-castrated, androgen-injected males had ovaries with an average weight of 16.6 mg.; the vaginae of all 7 test rats remained closed. The mean ovary weight of test-rats receiving pituitaries from castrated injected hosts was 22.8 mg.; the vaginae of 11 of the 15 test rats opened.

Although the gonadotropic potency of the pituitaries of both normal and castrated rats of both sexes was greatly reduced by administration of testosterone propionate, there still remained some capacity for gonad stimulation. The mean ovarian weight of the test rats receiving pituitaries from injected donors was 23.3 mg., whereas the mean ovarian weight of rats receiving no implantations was 12.7 mg.

It seems clear from these experiments that administration of the male sex hormone decreases the ovary-stimulating capacity of the anterior lobe in normal and in castrated male and female rats. Moore and Price (6) have demonstrated such a lessened capacity in young male rats, but they did not obtain a decrease in adult males with administration of 4 to 6 mg. of an-

drosterone for 20 days. The fact that injection of androgens does decrease the ovary-stimulating capacity of the anterior lobe is in accord with studies demonstrating that male hormone substances possess the capacity to prevent gonadal development in young rats (6, 8), and to interrupt the estral cycle in female rats (9, 10, 11) as well as the menstrual cycle in women (12). It is also in accord with studies suggesting that injections of androgens induce histologic changes in the anterior hypophysis (13, 14, 11).

This lessened capacity for gonad stimulation may be accomplished by the suppression of the production of the gonadotropic hormone, or by the release of the hormone from the anterior lobe. Both mechanisms may possibly play a rôle. Injection of the male hormone prevents the histologic changes that occur in the anterior lobe after castration (13, 14), and which are usually thought to be associated with the storage of gonad-stimulating substances. Also it has recently been reported that injection of sufficient amounts of testosterone propionate into normal female rats results in a certain degree of luteinization in the ovary (13, 11). This latter finding might support the possibility that injection of certain androgens may induce the release of a luteinizing factor by the anterior lobe under certain conditions.

SUMMARY

Administration of synthetic androgenic substance (500 gamma of testosterone propionate daily) reduces the gonadotropic properties of the pituitary, as shown by implantation of pituitaries from 82 androgen-injected and control male and female rats into 49 young litter-mate female rats. Hosts with pituitaries from testosterone-injected donors had an average of 23.3 mg. ovarian and 77.3 mg. uterine weight, and in some instances unopened vaginae. Hosts with pituitaries from control donors had an average of 65.0 mg. ovarian and 184.5 mg. uterine weight, and all cases had open vaginae. Although gonadotropic properties were greatly lessened by injection of testosterone propionate, there still remained some capacity of gonad stimulation, as seen in comparison with litter-mate hosts receiving no implants which had an average 12.7 mg. ovarian and 23.3 mg. uterine weight. The increase in gonadotropic capacity subsequent to castration, and augmented by the elapse of post-castration time, is largely but not completely negated by injection of testosterone propionate.

Discussion is given of the possibility that androgenic material can act to lessen gonadotropic substance, both by the suppression of production and by the release of pituitary-stored material.

REFERENCES

1. Smith, P. E.: Proc. Soc. Exper. Biol. & Med. **24**:131. 1926.
2. Smith, P. E., and E. T. Engle: Am. J. Anat. **40**:159. 1927.
3. Engle, E. T.: Am. J. Physiol. **88**:101. 1929.
4. Evans, H. M., and M. E. Simpson: Am. J. Physiol. **89**:371. 1929, *ibid.*, **89**:375. 1929.
5. Meyer, R. K., S. L. Leonard, F. L. Hisaw and S. J. Martin: Endocrinology **16**:655. 1932.
6. Moore, C. R., and D. Price: Endocrinology **21**:313. 1937.
7. Parkes, A. S.: Lancet **2**:674. 1936.
8. Hamilton, J. B., and S. L. Leonard: Anat. Rec. (To be published).

9. Robson, J. M.: Proc. Soc. Exper. Biol. & Med. **35**:49. 1936.
10. Nelson, W. O., and C. G. Merckel: Proc. Soc. Exper. Biol. & Med. **36**:823. 1937.
11. Wolfe, J. M., and J. B. Hamilton: Proc. Soc. Exper. Biol. & Med. **37**:189. 1937.
12. Hamilton, J. B.: Unpublished data.
Engle, C. T.: Am. J. Anat. **44**:121. 1929.
13. Nelson, W. O., and T. F. Gallagher: Anat. Rec. **64**:129. 1930, *ibid.*, Science **84**:230. 1936.
14. Wolfe, J. M., and J. B. Hamilton: Proc. Soc. Exper. Biol. & Med. **36**:307. 1937. Endocrinology **21**:603. 1937.

CHICK TESTIS WEIGHT RESPONSE TO GONADOTROPIC HORMONE

T. C. BYERLY AND W. H. BURROWS
From the Bureau of Animal Industry, U. S. Department of Agriculture
WASHINGTON, D.C.

For use as assay animals male chicks have the advantage of being relatively cheap and usually available. Moreover, they require a minimum of care and the equipment used is inexpensive. Quantitative measurement of the sensitivity of the chick testis to gonadotropic hormone will make possible its use in assays of gonadotropic potency.

The reports of numerous investigators have shown that the chick testis responds to administered gonadotropic hormone from various sources by rapid weight increase. The response to hormone from pituitary preparations and pregnant mare serum is known to be greater than that to hormone from pregnancy urine preparations. Asmundson, Gunn and Klose (1) and Breneman (2) have made recent reports of a qualitative nature, and these papers may be consulted for references to previous work.

MATERIALS AND METHODS

The chicks used were of various breeds and crosses. Male chicks were segregated by the so-called Japanese method (3). Sex-sorted chicks, especially of the Single Comb White Leghorn variety, may be obtained from many commercial chick hatcheries.

During the course of the tests recipient chicks were kept in ordinary commercial chick boxes at room temperature, or in the trays of a commercial incubator, without food or water. The unabsorbed yolk contained by the newly hatched chick will sustain life for 96 hours, after which time mortality from inanition begins. If chick boxes are used for holding chicks, each compartment must contain approximately 25 chicks in order to maintain proper environmental temperature.

Merthiolated mare serum[1] prepared and assayed at the University of California was injected subcutaneously into the cervical region of day-old male checks. Injected chicks and water-injected controls were killed 24, 48 or 72 hours after the time of injection, and the testes removed and weighed as quickly as possible after death of the chick. The necropsy of one chick required about a minute.

Other male chicks were used as recipients for day-old male chick pituitaries. The pituitaries were removed from day-old donor chicks, suspended in water

[1] Supplied through the courtesy of Dr. H. H. Cole. It was assayed on female rats 25 days old and was estimated to contain about 100 R.U. of gonadotropic hormone per cc.

and injected subcutaneously into the cervical region of the recipients. Total dosages of from 2 to 128 chick pituitaries were given. The higher dosages were necessarily given in split doses on successive days whereas some recipients of the lower dosages received a single injection as day-old chicks. The relative efficiency of single and divided doses can be judged only after the accumulation of more data.

The day-old chick pituitaries were implanted to obtain a measure of the gonadotropic hormone content of the recipient's pituitary, and to check similarity of response to hormone from pituitary and mare serum. Recipients were necropsied when 96 hours old, the testes removed and weighed. Water injected controls were used.

RESULTS

The data in table 1 show the numbers and average weights of testis pairs from control, mare serum-injected and male chick pituitary-injected male

TABLE 1. AVERAGE WEIGHTS OF TESTIS PAIRS FROM RECIPIENTS AND CONTROLS INJECTED WITH MARE SERUM OR MALE CHICK PITUITARIES. THE STANDARD ERROR OF EACH AVERAGE WEIGHT IS GIVEN

Mare serum recipients							Male chick pituitary recipients		
Dosage	24 hour		48 hour		72 hour		Dosage		
R.U.	Recipients	Av. wt. of testis pairs	Recipients	Av. wt. of testis pairs	Recipients	Av. wt. of testis pairs	Chick pituitaries	Recipients	Av. wt. of testis pairs
	no.	mg.	no.	mg.	no.	mg.	no.	no.	mg.
Control	20	7.2±0.47	20	7.0±0.38	82	6.1±0.18	2	1	7.7
0.5	—	—	—	—	7	5.7±0.57	4	4	7.6±1.95
1	12	8.8±0.43	9	9.8±1.23	41	8.2±0.39	8	5	9.8±2.71
2	7	10.0±0.25	7	9.6±0.98	40	9.7±0.56	12	1	11.8
4	5	9.0±0.45	5	9.8±1.75	46	9.9±0.58	16	13	11.9±1.26
8	7	14.3±2.18	5	10.2±1.66	29	12.6±1.05	24	2	8.6±1.65
10	14	12.8±1.51	15	13.1±0.88	78	11.4±0.35	32	16	14.4±1.46
12	—	—	—	—	8	13.6±1.97	48	4	6.6±1.30
16	5	12.4±1.63	6	12.7±1.25	30	14.1±0.92	64	17	15.6±1.92
20	4	8.8±0.75	4	12.3±1.65	38	13.8±1.16	96	7	17.0±1.58
25	—	—	—	—	10	16.5±1.74	128	4	18.5±3.81
32	5	14.0±2.60	6	16.2±1.21	26	17.2±1.28			
50	6	12.5±1.67	5	17.4±1.98	24	17.3±1.40			
100	—	—	—	—	15	22.7±2.29			
Total no. of recipients	65		62		392			74	

chicks. Testis weights showed considerable variability, but probably no more than the variability in weight of the 25-day rat ovary as indicated by the work of Cole (4) and Catchpole and Lyons (5).

The equation $\log y = A \log (x + C) + B$ . . . . . *(1)* was fitted to the data given in figure 1 for the 72-hour set of serum-injected chicks and their controls by the method of least squares after expansion of the equation by means of Taylor's theorem.[2] In equation *(1)* y is the weight of the average

[2] The authors are indebted to Mr. W. A. Hendricks of the Bureau of Animal Industry for advice and assistance in the mathematical treatment of the data.

testis pair at a given dosage; x is the dosage in R.U.; C is the potency in R.U. of the recipients' own pituitary, and A and B are constants determined by least squares. Constant A defines the slope; B is the value of log y when log $(x + C)$ is zero. In this analysis, the value of C was first estimated. The estimated value was used in the first fitting, and correction factors were obtained for A and C. These were applied, and the process repeated until the corrections for A and C were approximately zero. The value of C, determined in this manner, was about 0.5 R.U. The value of C obtained by fitting equation *(1)* to the 72-hour set of data was used in fitting the 24 and 48-hour sets of data.

TABLE 2. VALUES OF COSNTANTS A AND B, EQUATION (*1*), FOR THE OBSERVATION ON CHICK TESTIS WEIGHT AND A AND B, EQUATION (*2*), FOR THE 72-HOUR OBSERVATIONS OF COLE (4) ON 25-DAY RAT OVARY WEIGHTS, WITH THE STANDARD ERROR OF EACH VALUE

Source of data	A	B
24-hour chick	0.119 ± 0.0342	0.926 ± 0.0361
48-hour chick	0.181 ± 0.0222	0.898 ± 0.0233
72-hour chick	0.253 ± 0.0144	0.838 ± 0.0165
72-hour rat	0.301 ± 0.0397	1.245 ± 0.0678

The data for testis weights of recipients given male chick pituitaries, shown in table 1, were converted to a logarithmic basis, and a set of normal equations set up to determine the potency of the injected chick pituitaries in R.U. Theoretical values, read from the curve in figure 1, were used in these calculations. The values for 1-day-old male chick pituitary obtained in this way was 0.38 ± 0.057 R.U.

This value is somewhat lower than the estimate based on the controls and recipients for the mare serum, which was to be expected, since the pituitaries

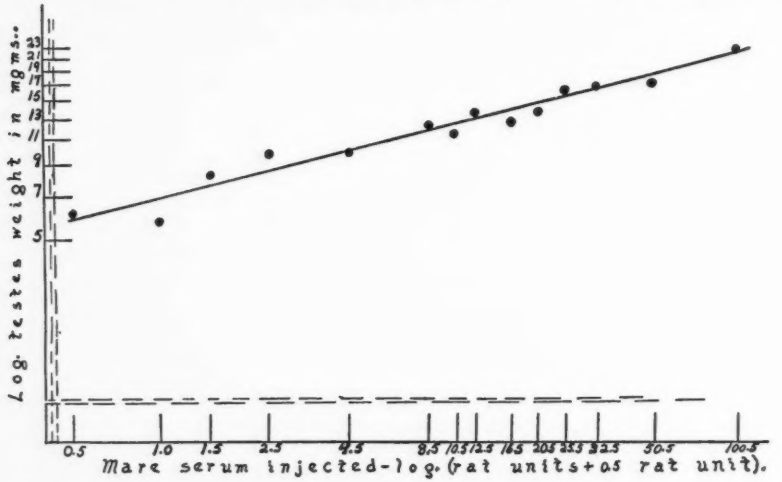

Fig. 1. Data for average weights of testis pairs from male chicks injected with mare serum as day-old chicks and necropsied 72 hours later, and their controls plotted against the dosage in R.U. + 0.5 R.U. The data are plotted logarithmically.

of the recipients were continuously active, and the method of administering the chick pituitaries was very crude.

It is suggested that 1 male chick unit of gonadotropic hormone be defined as that amount which, when injected subcutaneously into a male chick during the first day after hatching, will produce testes double the weight of control testes 72 hours after the time of injection. About 10 R.U. of mare gonadotropic hormone were required to produce testis pairs double the weight of the control testis pairs 72 hours after injection. One male chick U would thus be equivalent to about 10 R.U.

The values for constants A and B are given in table 2 with the standard error of each. Constant A is significantly larger for the 72-hour set of data than for the 24 or 48-hour set. This indicates a greater sensitivity of the 72-hour test. Constant B for the 72-hour set is smaller than B for the 24 or 48-hour set. This reflects the slight decrease in testis weight of the controls with age. The difference in value of constant B for the 3 sets of mare serum-injected chicks is not statistically significant.

Table 2 also contains values for A and B for the equation $\log y = A \log x + B \ldots\ldots (2)$ fitted by the method of least squares to the data given by Cole (4) in his table 2, for average ovary weights of rats necropsied 72 hours after the injection of mare serum. Y of equation *(2)* is average ovary weight at a given dosage; x is dosage in R.U. and A and B are constants with the same significance as A and B of equation *(1)*. The value of A for the rat data is insignificantly larger than A for the 72-hour chick series. However, allowance for the potency of the recipient rat's pituitary would increase the value of A in equation *(2)* so it is probable that the 25-day rat ovary is slightly more sensitive in its weight response to mare gonadotropic hormone than the chick testis.

SUMMARY

The newly hatched male chick testis responds to injection of mare gonadotropic hormone by rapid increase in weight. The response is probably a little less sensitive than the weight response of the 25-day rat ovary. Male chicks injected subcutaneously in the cervical region as day-old chicks, kept at 37.5°C. or slightly less, without food and water, and necropsied 72 hours after injection afford a moderately sensitive measure of gonadotropic hormone. Log testis weight plotted against log dosage shows a straight line relationship. The pituitary of the day-old male chick contains about 0.38 to 0.50 R.U. of gonadotropic hormone as assayed on day-old male chicks.

REFERENCES

1. Asmundson, V. S., C. A. Gunn and A. A. Klose: Poultry Sci. **16**:194. 1937.
2. Breneman, W. R.: Anat. Rec. **64**:211. 1936.
3. Jull, M. A.: Poultry Sci. **13**:250. 1934.
4. Cole, H. H.: Am. J. Anat. **59**:299. 1936.
5. Catchpole, H. R., and W. R. Lyons: Am. J. Anat. **55**:167. 1934.

EXPERIMENTALLY INDUCED OVULATION IN DWARF MICE[1]

CLINTON M. OSBORN
Biological Laboratories, Harvard University
CAMBRIDGE, MASSACHUSETTS

Hereditary dwarfism in mice was reported in 1929 by Snell (1), who described the dwarf characteristics and presented breeding data indicating that the mutation bred true as a Mendelian recessive. Snell concluded that both sexes were entirely sterile.

Studying the endocrine system of the dwarf mouse, Smith and MacDowell (2) confirmed Snell's observations on sexual infantilism and also described subnormal development of the thyroids, adrenal cortex, and anterior pituitary. By making fresh implants of rat pituitaries, they were able to correct some of the deficiency effects described. Growth was markedly stimulated, the thyroids looked normal, the adrenal cortex thickened, fertility was restored to some of the males, and definite corrective responses were noted in the female reproductive tract. The implants opened some of the vaginas, stimulated follicles to grow much larger than are typical of dwarfs, and produced corpora lutea. No mention is made of actual fertility in the females. According to MacDowell (3), ovulation does not occur in dwarf mice.

It is the purpose of this paper to report data on ovulation experimentally induced in female dwarf mice by using relatively pure gonadotropic fractions[2] of the anterior pituitary.

MATERIALS AND METHODS

The dwarf mice were secured by breeding heterozygous descendants from the stock originally brought to the Bussey Institute from England by Professor L. C. Dunn. The animals were allowed to remain with mothers until 35 to 40 days old. The mother was mated immediately after dropping each litter, and consequently successive litters were born every 23 to 26 days. This meant that each dwarf suckled through two periods of lactation. Dwarf mortality was thus minimized and the animals were healthy and vigorous when they were taken from their home cages. Injections were begun on one group of females at 56 days, and another group received first injections at 84 days. The FSH injections were administered twice daily for 4 days. Each animal of the younger group received a total dosage of 1.8 gm. equivalent[3] of FSH, while the older group was given a total dosage of 1.6 gm. equivalent per animal (table 1). These dosages were based on previous experience with FSH injections into dwarfs,

[1] Aided in part by a grant from the Rockefeller Foundation, administered by Frederick L. Hisaw.
[2] These gonadotropic fractions were kindly furnished by Dr. H. L. Fevold and Dr. G. Pincus and were fractionated according to the methods of Dr. Fevold.
[3] One gm. equivalent contained 2 R.U. of FSH as assayed on 21-day old female rats.

were found sufficient to grow several large follicles in the ovaries, and to increase the ovarian weight at least 100 per cent. All FSH injections were made subcutaneously. The FSH was pure enough to grow follicles without evidence of luteinization when injected into dwarfs for 5 consecutive days.

Each animal received only a single intraperitoneal injection of LH simultaneously with the last FSH injection. Three different dosages, 2 per cent, 2.5 per cent or 4 per cent of one R.U. of LH[4] were used (table 1).

TABLE 1. EXPERIMENTAL OVULATION IN DWARF MICE BY FSH AND LH INJECTIONS

Animal No.	Age at First Inj. days	Total FSH Inj., gm. eq. orig. powder	Total LH injected, % of 1 R.U.	No. eggs recovered	Remarks
107–2	56	1.8	4	0	Follicles not fully grown, probably not ready to ovulate.
115–1	84	1.6	4	2	Very small animal with delicate tubes. Other eggs may have been lost or destroyed.
115–2	84	1.6	4	0	Three follicles had hemorrhaged; others showed some luteinization.
100–1	56	1.8	2.5	0	Histological sections showed several very large follicles apparently ready to rupture. More time or LH perhaps necessary.
148–1	56	1.6	2.5	3	Two other large follicles showed some luteinization.
(60) 122–1	84	1.6	2.5	3	
(56) 127–1	84	1.6	2.5	7	
191–1	56	1.8	2	4	
191–2	56	1.8	2	3	Two other large follicles apparently ready to rupture.
28–1	84	1.6	2	8	
28–2	84	1.6	2	2	Some undoubtedly lost, due to a break in the tubules.
31–1	84	1.6	2	4	

Ovulation was determined by the actual recovery of eggs from the tubes following the method of Lewis and Wright (4). This was considered the only dependable technic, because the ovaries are so small that methods commonly used (counting points, etc.) are unsatisfactory. The animals were autopsied and tubes searched for eggs 15 to 30 hours after the last injection. Ovaries and uteri were fixed and serially sectioned.

[4] One R.U. of LH is the amount necessary to increase the weight of the seminal vesicles of a 21-day old male rat about 100 per cent (5).

OBSERVATIONS

As may be seen in table 1, eggs were recovered from 9 of 12 animals injected. From the 9 ovulating animals, a total of 36 eggs was definitely recovered, averaging 4 eggs per animal. Some ova were undoubtedly lost or destroyed. Dwarf ovaries are much smaller than those of normal mice and the number of developing follicles is less; consequently FSH treatment never develops extremely large ovaries in dwarfs, even though the follicles present apparently give a maximum response. This initial deficiency in number of follicles directly limits the number of eggs to be ovulated.

DISCUSSION

When Smith and MacDowell (2) studied the endocrine glands of the dwarf, they were impressed by the similarity between dwarfs and hypophysectomized animals. However, the gonads did not show degeneration typical of hypophysectomy. The testes were firm, and considerable numbers of apparently normal motile sperm were found in the epididymis. The ovaries, although very small, were healthy in appearance and contained follicles much larger than are typical of hypophysectomized animals. These obvious differences with regard to the gonads led Smith and MacDowell (6) to test the dwarf anterior pituitary for gonadotropic activity. They concluded that the dwarf pituitary contained more gonad-stimulating hormone per unit quantity of tissue than the normal mature female mouse pituitary. They studied only the gross gonadotropic activity of the dwarf pituitary and made no attempt to separate physiologically its follicle-stimulating and luteinizing components.

If the dwarf pituitary per unit weight is normal, or above, in gonadotropic potency one might expect the reproductive system to be more highly developed than it actually is, although the dwarf anterior pituitary is small in proportion to the animal's body size (6). That the ovary is subnormally developed may be explained in one of two ways: either the gonads are relatively insensitive to pituitary stimulation, or the gonadotropic output from the pituitary is insufficient or incorrectly balanced with respect to follicle-stimulating and luteinizing components. That dwarfs' ovaries are somewhat less sensitive to FSH stimulation than are the ovaries of normal animals is indicated by the fact that two to three R.U. are needed to double the weight of the ovary. Furthermore, the responses of FSH treated dwarf ovaries exhibit considerable variation. They occasionally grow but slightly, even when subjected to 4 or 5 R.U. The unresponsiveness of the dwarf ovary is correlated with the development of only a few follicles, and thus limits the number of eggs ovulated.

The accessory organs seem quite sensitive. Balloon uteri are frequently produced where ovaries remain superficially unstimulated. Vaginas, if not already open, almost invariably do so after 2 or 3 days of FSH treatment.

The dwarf ovary is apparently very sensitive to LH stimulation, and luteinizes readily. Many follicles luteinize without ovulating. Hemorrhagic follicles are very common where slight traces of LH are present in the FSH preparations. Luteinization does not occur normally in the dwarf ovary. Many dwarf ovaries have been examined as controls, but no corpora lutea have been

found. Hemorrhagic follicles without evidence of luteinization have been found twice in unilaterally ovariectomized animals, but at present it cannot be stated whether this was due to release of LH from the animal's own pituitary, or to some abnormality.

Experimental ovulation by a method similar to that described here has been reported (7) in anestrous cats and hypophysectomized rabbits (8). These investigators succeeded in producing superovulation, and in some instances as many as 3 to 5 times the normal number of ovulation points were recorded. However, it is interesting to note that, as time elapsed following hypophysectomy, fewer ovulation points were obtained. This condition may roughly parallel the state of the dwarf ovary and provide additional evidence for the failure of superovulation in the dwarf mouse. Possibly, the reason why the optimum ovulation dosage was not found for these animals was because such experiments must necessarily be limited to small numbers of dwarfs due to the breeding problem.

SUMMARY

Female dwarf mice are sterile with sexually underdeveloped reproductive tracts. The ovaries, however, show far more follicular differentiation than is typical of hypophysectomized animals. Ovulation does not occur normally, and consequently corpora lutea are never developed.

Experimental ovulation averaging 4 ova per animal was produced in 9 of 12 dwarf mice by growing the follicles with FSH (subcutaneous injections) followed by 2 to 4 per cent R.U. of LH (injected intraperitoneally) at the time of the last FSH injection. Possible explanations for failure to obtain ovulation of large numbers of eggs are suggested.

REFERENCES

1. Snell, G. D.: Proc. Nat. Acad. Sc. **15**:733. 1929.
2. Smith, P. E., and E. C. MacDowell: Anat. Rec. **46**:249. 1930.
3. MacDowell, E. C.: Carnegie Institution of Washington, Year Book No. 30, p. 54. 1931.
4. Lewis, W. H., and E. S. Wright: Contributions to Embryology, No. 148, Publication No. 459 of Carnegie Institution of Washington, p. 113. 1935.
5. Fevold, H. L.: Annual report of the Station for Experimental Evolution. Cold Spring Harbor, N.Y., Vol. 5, 1937.
   Fevold, H. L., F. L. Hisaw and S. L. Leonard: Am. J. Physiol. **97**:291. 1931.
6. Smith, P. E., and E. C. MacDowell: Anat. Rec. **50**:85. 1931.
7. Foster, M. A., and F. L. Hisaw: Anat. Rec. **62**:75. 1935.
8. Foster, M. A., R. C. Foster and F. L. Hisaw: Endocrinology **21**:249. 1937.

CASE REPORTS

THE CUSHING SYNDROME

CHARLES WILLIAM DUNN
From the Graduate School of Medicine, University of Pennsylvania
PHILADELPHIA, PENNSYLVANIA

In 1932, Cushing (1) reported 12 cases with an endocrine syndrome consisting of a rapidly acquired regional obesity, hypertension, hypertrichosis, genital dyscrasia, physical asthenia, plethora, purplish striae atrophica, and osteoporosis resulting frequently in kyphosis and shortened stature. He had observed 3 cases personally; the others were obtained from the literature, classified therein as polyglandular disturbances.

Study by serial section of the anterior pituitary disclosed, in each current case, an unsuspected adenoma of the basophile cells, a lesion originally described by Erdheim (2) in 1903, but which he did not consider pathological in his cited cases. Cushing interpreted the basophilic adenoma as the primary pathology in the endocrine syndrome, naming it pituitary basophilism; the associated adenoma or hyperplasia of the adrenal cortex he believed to be due to the excessive adrenotropic factor: thus his pathophysiological conception of pituitary basophilism was that of a hyperbasophilic state, on the order of hypereosinophilism, as in acromegaly.

In basophilism, the ovaries are often found to be small with atretic follicles, or large from luteo-cystic masses; the uterus is usually atrophied; the testes are small or normal in size, with histological changes indicating lowered functional activity. The thyroid is enlarged from colloid changes, atrophied or normal, and the parathyroid glands normal, or exhibiting capillary dilatation, adenomatous formation associated with fibrotic, or fatty infiltration.

The kidneys, clinically and grossly normal, reveal microscopic evidence of mild nephritis, although in subsequent case reports they exhibited nephrosclerosis and stones. Osteoporosis has occurred from a mild to an extreme state, involving chiefly the spine, ribs, and skull. The large blood vessels show arteriosclerosis, and the heart ventricles are hypertrophied.

The disorder usually affects young adults and has, in the majority of cases, resulted in death within 5 years of its onset, although Cushing (1) and Rolleston (3) have reported cases living 10 or more years after the appearance of the symptoms. The verified cases have been observed for only a short period, as an intercurrent infection, usually pneumonia, has generally caused death.

Cushing recommended a re-examination of the available specimens from the cases formerly termed polyglandular, and the new investigations con-

firmed the presence of basophilic adenoma of the anterior lobe and comparable adrenal cortical lesion. Whereas this strengthened his theory of the basophilic adenoma as the primary factor, he warned against unquestioning acceptance of it. This caution was justified, as forthcoming evidence was to prove the Cushing syndrome to be of a varied primary origin.

Leyton (4) found a cause to be carcinoma of the thymus (in a child and adult male), which he reported under the title 'Multiglandular Disease,' Kepler (5) later corroborating it. Carcinoma of the adrenal cortex was observed by Hare (6), Lyall (7), Kepler (5), Cahill (8), and others. In both these causal conditions, section study of the pituitary gland showed no adenoma or numerical shift of the basophile cells. That the primary origin of the Cushing syndrome varies has been testified by MacCallum's report (9) of an adenoma of the intermediate lobe (the pathological diagnosis of which Rasmussen (10) disputes, holding this basophilic formation to be a normal manifestation), Fuller's and Russell's case (11) of chromophobe adenoma of the anterior lobe in a young male, Cohen's report (12) of carcinoma of the anterior pituitary lobe, and Norris' (13) report on arrhenoblastoma of the ovary. This evidence, together with its occurrence in benign and malignant form, place a profound responsibility upon the diagnostician.

Malignancy of the adrenal cortex is not always associated with the Cushing syndrome. Ullom (14) reports another case of adrenal cortical carcinoma, in which the autopsy findings give no signs or symptoms of Cushing's syndrome.

An observation clarifying the pituitary pathology of Cushing's syndrome was made by Crooke (15) in 1935. It was confirmed by Rasmussen (10) and recently made again by Crooke (15) in the pituitary carcinoma of Cohen (16). He found that in Cushing's syndrome, regardless of its form of primary origin, there occurs a loss of basophile granules with hyaline cytoplasmic changes and frequent nuclear mitotic activity of the basophile cells. Crooke (15) has observed these cellular changes in over 29 cases, and declares them to be hyperfunctional. As the basophile cells are the alleged source of the gonadotropic fraction, a paradox is created when a primary physiological cellular hyperfunction is reputed, yet clinical evidence and gonadal pathology exhibit a hypofunctional state.

In a letter of May 21, 1937, Rasmussen (17) informs me that the most clear-cut example of the Crooke reaction that he has observed was in Norris's (13) case of arrhenoblastoma of the ovary; furthermore, the pituitary glands from 10 cases of Cushing's syndrome showed the same reaction, but disclosed only one basophilic adenoma. Kessell's review (18) of the literature on Cushing's disease revealed just 35 cases coming to necropsy, in 21 of which basophilic adenoma was discovered.

Grollman (19) states that the adrenal cortical cells are not involved in Cushing's or the adrenogenital syndromes, nor in virilism; that prolonged administration of adrenal cortical hormone has had no effect on the reproductive system; that the reproductive changes in Addison's disease are due to the secondary pituitary pathology; and that adrenalectomized animals, which are stunted and in continual diestrus, show growth and estrus after receiving pituitary implants but not after injections of adrenal cortical hormone. He divides the adrenal cortex into two parts, the true cortex tissue and the androgenic, and believes the latter is involved in the above syndromes. The androgenic tissue, which lies between the cortical reticular layer and the medulla of the adrenal gland, is especially prominent in the fetus and during the first year of life; it hypertrophies at puberty, during pregnancy, and after castration in the male. It is also found in the so-called accessory adrenal bodies, and as intra or para-gonadal cell masses.

Broster and Vines (20) report, besides an androgenic principle in the urine of patients with adrenogenital syndrome, a new biochemical compound that Marrian and Butler state has the chemical formula $C_{21}H_{36}O_3$ and which appears to be specific to virilism since it is not present in the urine of normal men and pregnant and non-pregnant women, and disappears after unilateral adrenalectomy has been performed in the cases of adrenogenital syndrome.

Walters (21), Broster (22), Cahill (8), and others have indicated that the surgical problem of adrenal carcinoma is compounded and complicated by primary extension of the growth or by its metastases. The basophilic adenoma, often of microscopic size and indifferent location, in the anterior lobe causing this syndrome are reasonably beyond surgical acumen. Radical enucleation of the anterior lobe will induce an equally fatal condition, Simmonds's disease. The neuro-surgeon is very reluctant, and justly so, to operate upon the pituitary gland except when malignant or adenomatous tumors produce pressure on the optic nerve or when the clinical signs indicate that surgery is required.

The following forms of therapy giving good results in cases of pituitary basophilism have been reported; x-ray therapy to the pituitary, by Cushing; bilateral adrenal denervation, by Crile (23); unilateral adrenalectomy in malignancy of the adrenals, by Walters (21); and surgery of the anterior pituitary lobe, by Lisser (24). There was a recurrence of the basophile syndrome in both Lisser's and Walters' cases, following excellent post-operative results. The cause of recurrence in Walters' case was the malignant factor, but in Lisser's case the cause was unknown. X-ray therapy has not shown consistent therapeutic effects, an observation that is confirmed in 3 of our cases.

In cases of verified and unverified Cushing syndrome, virilism, adrenogenital syndrome, diabetes of bearded women, and arrhenoblastoma of the ovary, these clinical data suggest that this group of endocrine disorders might be classified as benign and malignant forms of an androgenic-pituitary syndrome.

The features previously mentioned usually comprise the Cushing syndrome. However, Dr. Cushing also notes less common features, namely, anxiety state and suffocative spells; head, orbital, neuralgic, lumbar, referred and abdominal pains; exopthalmus, various visual disturbances and retinal hemorrhage; purpura-like ecchymosis, acne, pigmentation, reddish striae; polyuria and nocturia; edema of the lower extremities; and a tendency to pneumonic infections.

Analysis of the symptoms observed in basophilism reveals a majority of them in the various endocrine syndromes that are pre-eminently hypogonadal.

REPORT OF CASES

This report concerns a group of 11 cases showing regional obesity, increased systolic pressure, hypertrichosis, asthenia, physical incapacity, pains common to basophilism, purpura-like ecchymoses and striae of a reddish or purplish color. The individuals are short in stature and round-shouldered, with a dusky, reddish and plethoric countenance, definite signs of genital dystrophy, and symptoms of the hypo-ovarian syndrome. A primary hypogonadal state has been reasonably excluded, as well as non-endocrine causes of hypertension.

In 1933, 4 cases presenting x-ray and clinical evidence of pituitary lesions (2 of chromophobe adenoma, and *cases 4* and *8* with basophilic adenoma) were given x-ray to the pituitary. Convulsions were present in 1 case of each type. Excellent therapeutic results were obtained in the chromophobe adenoma cases; and whereas good early results were exhibited in the basophilic cases, as evidenced by the decreased incidence of convulsions in *case 8*, (relief from head pains, reduction of hypertension, and amelioration of the incapacity), there was no effect on the obesity or the hypertrichosis; moreover, the relief was only temporary, and the original clinical picture returned again after the second series of x-ray treatments. Further x-ray therapy was deemed inadvisable by the roentgenologist.

Case 4 especially suffered severe migraine attacks from 12 to 24 hours after each radiation, and in the second series she was given from 10,000 to 50,000 I.U. of progynon-B 4 hours before each radiation. This was found to allay or abort, depending upon the dosage and amount of radiation given, the post-radiation head pains. As *case 4* continued to have progressive clinical signs (increasing severity and incidence of head pains, rising hypertensive level, increasing hypertrichosis and obesity, and diminishing menses) 50,000 I.U. progynon-B was administered bi-weekly to control the migraine. After 6 months therapy, control of the symptoms and marked general improvement was observed. This therapy, continued in *case 4* and extended to other cases, seems to be vouched for by the following factors.

a). Estrin injections prevent the appearance of basophile castrate cells in the pituitary of castrated animals. *b*). In 1935, upon noting the similarity between the castration and Crooke reaction basophile cells, Nelson (25) stated, "On the basis of the foregoing experimental evidence it is interesting to speculate upon the possible value of estrin therapy in the treatment of pituitary basophilism—if a casual relation actually exists between basophilism and the clinical sign, it is entirely conceivable that an alleviative effect might be secured through properly controlled estrin injections." *c*). Zondek (26) in 1936 reported that the function and size of the pituitary gland in rats were inhibited by high-dosage estrin injections. *d*). The cause-and-effect theory as stated by Grollman (19) in his pituitary-ovary and androgenic complex conception of the Cushing and adrenogenital syndrome. *e*). The author's report (27) in 1934 on the effect of estrin in migraine and the hypo-ovarian state, also the results of therapy in blocking the x-ray radiation attacks of migraine. *f*). An antagonism between the corpus luteum hormone and the adrenal cortex or the androgenic zone, as indicated by the results observed with proluton and ovarian concentrate therapy, in cases exhibiting obesity, hypertension, hypertrichosis, dysmenorrhea, acne, and rough oily skin.

Before therapy was instituted, the possibility of a malignant type of Cushing syndrome was considered in all the cases, and reasonably excluded. It is conceded that no verified case of Cushing syndrome is presented; nevertheless, it must be noted that only the 2 untreated cases died (August 1936), from a pneumonic infection.

Case 1, a pupil nurse, 21 years old, was referred by Dr. J. T. Beardwood Jr., in

Nov. 1935, because of physical incapacity during the preceding 4 months. She had a systolic pressure of from 160 to 180 and a diastolic of 90 mm. Hg, for which no cause could be found. There had been a sudden gain in weight during the previous year, and the headaches, begun in July 1935, were becoming very frequent and of the severe migraine type. She complained also of suffocative attacks, palpitation, recurrent pain in the right lower quadrant and upper lumbar region, severe facial neuralgia, and abnormal hair growth. About 4 months previously she had first observed hair growth on the upper lip and sides of the face, and just a month before that hair had appeared on the breasts, abdomen, and lower extremities.

At the menses, which were diminishing, she was confined to bed because of the intense head and abdominal (R.L.Q.) pains; also at this time the right breast became nodular and painful, and acne of the face, chest, and back appeared.

Other physical features were the reddish dusky facies, and moderate obesity except in the extremities. The eyes were prominent, the thyroid was enlarged, the right breast showed mazoplasia, and the skin of the face was warm and oily, whereas that of the legs was dry, cold and bluish.

Results of a complete examination of the blood and urine were negative, except for the blood chlorides which were 616 mg. per cent. The sugar tolerance test showed a diabetic curve, and x-ray examination showed osteoperosis of the cervical vertebrae.

From 10,000 to 50,000 I.U. of progynon-B, and from 1 to 0.20 I.U. of proluton were administered 2 to 3 times a week. Although she had been confined to the hospital for 4 months without gaining relief and in a progressive symptomatic state, discharge from the hospital was possible in 6 weeks. The head pains were under control, and the menses improved. The systolic pressure was reduced to between 130 to 140 mm. and the diastolic to between 74 and 90 mm. Hg, at which level it was stabilized, after 0.5 I.U. of proluton was given every fifth day for 2 months. During the next 2 months she received 25,000 I.U. of progynon-B weekly, after which therapy was discontinued.

Two months later she was still symptom-free, the systolic pressure was 130 mm. Hg, and she had returned to training. There was an early gain in weight of 16 lb. and a subsequent loss of 20 lb., the hypertrichosis had diminished, and the improved menses can be directly attributed to the therapy. The blood chlorides were reduced from 616 to 436 mg. per cent of blood, and the sugar tolerance curve changed from a diabetic to a flat type of curve.

Case 2, a single woman, 24 years old, was referred by Dr. F. W. Smith, in December 1935, because of severe headaches, physical incapacity, and rapid regional weight gain during the preceding year. One year previously there had appeared polydipsia, polyphagia, polyuria, and nocturia; later, there occurred severe attacks of epigastric pain, and in the last half of the year, acute attacks of fronto-orbital pain and facial neuralgia. She had gradually become weakened, irritable, nervous, and extremely depressed; she slept poorly, and more recently there had been marked dyspnoea and suffocative attacks. The menses had greatly diminished, she complained of hot flashes, increasing head pains, acne of the face, back, and chest, and had observed reddish striae on her abdomen, back, thighs, and breasts.

The physical examination showed a reddish plethoric face, and obesity except in the extremities. The eyes were prominent, the thyroid was palpable, the breast parenchyma diminished, the systolic pressure was 148 and the diastolic 78 mm. Hg. The skin of the face and body was oily and moist, whereas that of the legs was dry and of a bluish color and purpuric-like ecchymotic areas were present. There was hair growth on the face, upper lip, breasts, abdomen, and extremities. The only positive

blood study was a blood chloride of 363 and 416 mg. per cent. X-ray examination showed a minor decalcification of the occipital bone.

She was administered 300 B.U. of progynon-DH and 4 ovarian concentrate capsules daily for 2 months. However, this did not control conditions, and her weight rose from 133 to 150 lb. The striae increased, the menses became scantier, and weakness, depression, hot flashes, and hypertension remained uninfluenced. The dosage was increased to 1500 B.U. of progynon-DH and 8 capsules daily of ovarian concentrate. After 6 months of increased dosage she was in good condition, with her weight at 139 lb., the systolic pressure at 108, and the diastolic at 60 mm. Therapy was discontinued 3 months later.

In May, 1937, she was in excellent health, and employed. She weighed 109 lb., the systolic pressure was 108 mm. Hg, the menses were normal, there had been no increase in the hypertrichosis, and the purplish striae had disappeared (fig. 1).

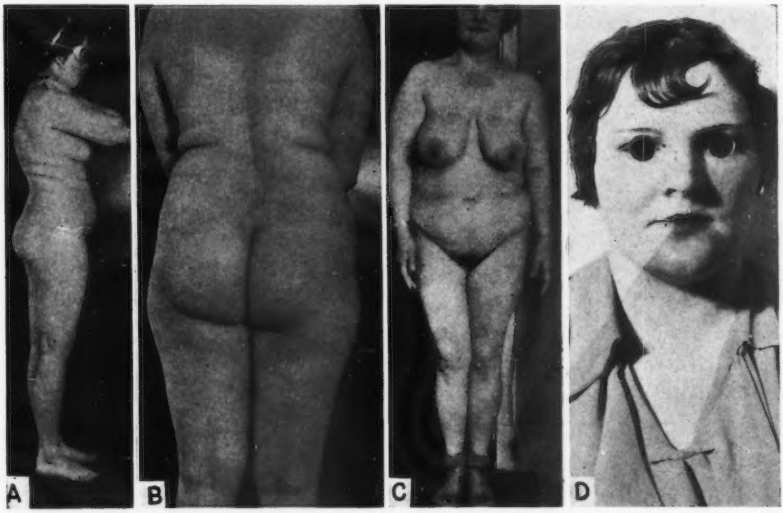

Fig. 1. *A, B. Case 2.* Note regional obesity, striae and acne. *C, D. Case 6.* Before therapy, and after 1 year of inadequate estrin therapy. Note the development of facial plethora associated with general progression of symptoms.

Case 4, a para I, 28 years old, was first examined in May 1932, when 22 years of age. At the age of 21 she had gained weight rapidly, except in the extremities, and had had amenorrhea for 6 months, followed by a pregnancy that terminated in a normal delivery on Jan. 1, 1932. During the preceding trimester the systolic pressure was 140 mm. She had been suffering severe migraine, and had observed growth of hair on her face and body.

When first examined, 4 months post partum, she complained of weakness, nervousness, and depression; migraine, facial neuralgia, blurring of vision and temporary blindness; suffocative attacks, hot flashes, attacks of abdominal distension and increasing obesity; acne, spontaneous ecchymosis, increasing coarseness of the skin and increasing hypertrichosis on the face and body. The blood pressure was normal.

In Oct. 1933, she returned because of a continuing gain in weight, increasing severity of the migraine, and progressive hypertrichosis (the facial hair having to be clipped once a week). At this time the systolic pressure was 150 mm., and x-ray

treatments to the pituitary were ordered. This relieved the headaches and reduced the blood pressure to normal, but the obesity remained unchanged. In 2 months the symptoms recurred, the blood pressure again rose to 150 mm., and the weight gain continued.

As a migraine attack occurred about 12 hours after each x-ray treatment, in the second series of x-ray treatments 50,000 I.U. of progynon-B was administered 4 hours prior to radiation, which either blocked or minimized the migraine attack. The therapeutic effect of the second series of x-ray corresponded to that of the initial series, but when the symptoms again recurred, further x-ray treatment was advised against, inasmuch as total x-ray dosage had been used.

Since morphine and the barbiturates gave no relief from the head pains, and progynon-B had usually aborted the x-ray induced migraine attacks, 50,000 I.U. of progynon-B was administered twice weekly. Within a few months, this had produced allay or control of the migraine, and had reduced the systolic pressure to normal. Subsequently, the migraine attack came only premenstrually, at which time it was also found that the systolic pressure rose.

This patient and *case 3* stopped treatment after the symptoms were relieved and the weight gain had been controlled without dieting. After 2 months, both patients

Fig. 3. *Case 10. A,* May 1936, before therapy. *B,* November 1936, after 6 months of therapy. *C,* May 1937, after 1 year of therapy.

returned because of the reappearance of the migraine. It was then found that the systolic pressure had risen; the menses had diminished or ceased; the breasts, which the estrin stimulation had enlarged, were again hypoplastic; and the hair growth, which had been controlled, was increasing and extending. One to 2 I.U. of proluton was then administered twice weekly for 3 weeks. This did not relieve the migraine, but reduced the systolic pressure from 148 to 118 and the diastolic from 90 to 70 mm. Hg.

This patient (*case 4*) has been observed for 5 years, during which time there have been lapses in therapy. With regular weekly therapy of progynon-B, alone or combined with proluton, there has been control of her weight gain, loss of plethora, approximately 70 per cent loss of the hypertrichosis, and control of the blood pressure at normal levels. Amenorrhea persists unless progynon-B and proluton are given in series, after the method of Kauffman.[1]

Case 5, a para X, 42 years old, was referred in Oct. 1935, because of enlarged

[1] The patient's last menses occurred in July, 1936. A Friedman test, March 20, 1937, was negative. In July, 1937, the physical examination and roentgenogram showed a 6-months' pregnancy. During the last 6 months she has required much less progynon-B and has been in excellent condition, for which the high level of blood estrin during pregnancy is probably accountable. On Oct. 25, 1937, a normal child was born; labor was induced because of a rise of systolic pressure to 150 mm.

sella turcica, hypertension (a systolic pressure of 220 and a diastolic of 130 mm. Hg), profound weakness, depression, severe migraine, hot flashes, vertigo, palpitation, suffocative attacks and dyspnoea, lumbar pains, polyuria, edema of the legs, gain in weight, and the loss of scalp and pubic hair. The head pains had appeared following her pregnancy in 1933, and were increasing in frequency and severity. The menses were alleged to be normal.

The physical examination revealed thin scalp hair, plethoric facies, bright prominent eyes, and an enlarged thyroid. The heart was also enlarged, the abdominal contour was that of a pregnant woman, the legs were obese, which however, was due to edema. Purplish striae were present on the lower abdomen and inguinal region, and there was hair on the face, upper lip, and abdomen.

The blood chemistry and urine studies were normal. The B.M.R. was +48 and 18. X-ray examination showed a unilateral erosion and general enlargement of the sella turcica, and decalcification of the dorso-cervical vertebrae.

She was given 50,000 I.U. of progynon-B in order to demonstrate the usual relief of the migraine and the associated symptomatology, as well as the lowering of the hypertension; ovarian concentrate was administered when the systolic pressure was 180 mm., which further lowered the blood pressure to 160 mm. At this point, edema of the lungs occurred and therapy was discontinued. She was hospitalized for 1 week, was discharged in good cardiac balance, and kept under clinical observation until her death, from pneumonia, in August 1936.

Case 6, a non-parous woman, 23 years old, was referred by Dr. H. G. Williams. Her menarche occurred at 9 years, the early menstrual periods came every 2 weeks, were copious, and lasted from 7 to 10 days. She stopped growing when 13 years of age, at about which time she complained of polyuria and nocturia. When 18 years old, her periods diminished from 10 days to 4, she began gaining weight rapidly, and during the preceding year she had commenced having headaches, palpitation, suffocative attacks, weakness, and periods of temporary blindness. These attacks had increased in severity to a degree that incapacitated her at the time of examination.

During the previous few months, the menses had been scant and for 2 days only, and hot flashes, irritability and depression had appeared. The headaches, at this time more severe and of longer duration, were often associated with severe facial neuralgia, muscular twitching, lumbar pain and abdominal distension. The patient had observed an increase of hair growth on the face and extremities, and a falling out of scalp hair; she stated that her skin had become coarser and bruised easily, and that facial acne was of recent origin.

The physical examination showed a moderately full face of a reddish dusky color, the eyes prominent, and the thyroid palpable. The heart and pulse rate varied from 80 to 100. The systolic pressure was 130 and the diastolic 84 mm. Hg. The breasts and abdomen were obese, the extremities normal. There were numerous white striae on the abdomen and thighs, moderately fine hair on the face, and coarse hair on the lower extremities. The positive laboratory finding was a flat sugar tolerance curve.

A hypo-ovarian state could have accounted for the clinical symptoms, but early basophilism was considered. The patient was given from 300 to 1000 B.U. of progynon-DH daily, which reasonably controlled conditions and improved the menses during the 18 months it was administered.

There was, however, a general progression of signs; the blood pressure gradually rose, the obesity increased, marked plethora developed, the migraine attacks became frequent at premenstruation, and by April, 1937 she presented a reddish plethoric facies, had a systolic pressure of 154 mm., was very depressed and weak, and incapacitated by the migraine and facial neuralgia. The menses were delayed.

She was then administered 50,000 I.U. of progynon-B, twice weekly; after the third dose, relief was present and the systolic pressure was down to 130 mm. Intensive therapy is being maintained, the plethora of the face is receding, and relief of symptoms and general improvement has occurred (fig. 1 *D*).

Case 10, a para II, 37 years old. Her menses started at 13 years of age, then appeared irregularly from 2 to 6 months, and lasted 6 days. The flow was profuse, and accompanied by left ovarian pain. At 16 years of age, hair began to grow on her face, continuing until she was 18 years of age, when x-ray and radium therapy was used for the facial hypertrichosis. This resulted in telangiactasis and scarring. Following a pregnancy in 1925, the menses became regular for a short time, but the amenorrhoeic periods recurred. She received theelin injections, and later x-ray therapy to the pituitary, but neither helped the amenorrhea.

In 1932, head pains appeared. These increased in severity at menses, disappeared during her second pregnancy, only to reappear again after her delivery, on Feb. 25, 1936. During the second trimester, she noticed abnormal hair growth on her body and extremities, which continued increasing to date of examination, May 1936.

One month post partum she became very weak, nervous, depressed, and complained of hot flashes. The headaches became constant, the recent plethora of her face and the gain in weight continued, and palpitation, suffocative attacks, sleeplessness, polyuria, and pain in the adrenal regions had incapacitated her.

The physical examination showed a moderately short, obese woman. The facial features were distorted by the plethora, the eyes were dull and depressed, and the thyroid was palpable. The breasts were obese, the heart rate was rapid, the systolic pressure 158 and the diastolic 90 mm. Hg. The abdomen was obese, and covered with hair, which continued down over the lower non-obese extremities, the upper extremities were also non-obese and hairy, the clitoris was normal in size, and the uterus of post partum size. The pubic, vulvar and perineal regions were covered with coarse, heavy hair.

As she was in an acute state of physical and mental breakdown, therapy was immediately instituted without laboratory investigation. 50,000 I.U. of progynon-B was administered 2 to 3 times a week for 5 months (fig. 2 *B*), and during the succeeding 5 months 50,000 I.U. of progynon-B and 1 to 2 I.U. of proluton in series.

After 10 months the symptoms were under control, there was a loss of 30 lb. in weight, regular menses, and normal blood pressure (fig. 3). Premenstrually there is a tendency for the blood pressure to rise and the migraine to appear, but this is controlled by increasing the dosage of progynon-B[2] (fig. 2 *C*).

DISCUSSION

In a group of 11 cases (women) which, pathologically speaking, have been defined as a benign form of Cushing syndrome, the administration over a period of 1 to 4 years of large doses of progynon-B and progynon DH, alone or in combination with proluton, has produced measurable therapeutic results. It has effected the disappearance of the plethora and the loss of weight without diet regulation, the reduction of the hypertension and hypertrichosis, the improvement and disappearance of the genital dystrophy, and the relief of the incapacitating symptoms, all features characteristic of pituitary basophilism.

[2] I wish to thank Dr. Gregory Stragnell, Schering Corporation, for the generous supply of progynon-B and proluton administered to these cases, and to Dr. Frederic Fenger, Armour and Company, for the ovarian concentrate.

The duration of therapy requisite to effect clinical results appears to be proportionate to the duration of the syndrome prior to the institution of this therapy. The sooner progynon-B therapy is begun, after the onset of the syndrome, the more favorable is the prognosis and opportunity to prevent secondary non-endocrine pathology.

Breast parenchymal hypoplasia is present in these cases, and it reacts characteristically to the repeated large dosage of estrin by becoming firm and sensitive, at which time there is general relief of symptoms, to be fol-

TABLE 1. SUMMARY OF THE FINDINGS IN 11 CASES OF CUSHING SYNDROME

Cases	1(AH)	2(EW)	3(MB)	4(FK)	5(LP)	6(CB)	7(AW)	8(HJ)	9(DB)	10(ST)	11(SB)
Regional Obesity	x	x	x	x	x	x	x	x	x	x	x
Statural Changes	x	x	x	x	x	x	x	x	x	x	x
Sexual Dystrophy	x	x	x	x	x	x	x	x	?	x	x
Hypertension	x	x	x	x	x	x	x	x	x	x	x
Hypertrichosis	x	x	x	x	x	x	x	x	x	x	x
Asthenia	x	x	x	x	x	x		x	x	x	x
Osteoporosis	x	x	x	x	x						
Suffocative Attacks	x	x	x	x	x	x			x	x	
Polyphagia Polyuria		xx			xx	xx			xx		xx
Acne	x	x	x	x	x	x	x	x	x	x	x
Pigmentation	x	x	x	x	x		x	x		x	
Dry Skin	x	x	x	x	x	x	x	x	x	x	x
Striae	R	R	P	P	P	P	W	W	W	W	
Purpura-like Ecchymosis	x	x	x	x	x	x	x	x	x	x	x
Viscual Disturbances	x	x	x	x	x	x			x		
Lumbar Pain	x	x	x	x	x	x	x	x	x	x	x
Abdominal Pain	x	x	x	x		x	x		x	x	x
Orbital Pain	x	x	x	x	x	x	x	x	x	x	x
Head Pain	x	x	x	x	x	x	x	x	x	x	x
Neuralgia	x	x	x	x	x	x			x	x	
Abdominal Distension	x	x	x	x	x	x		x	x	x	x
Hypo-ovarian Symptoms	x	x	x	x	x	x		x	x	x	x

lowed shortly by the reappearance or improvement of the menses. If estrin therapy is then diminished or discontinued for 4 to 8 weeks, the breast hypoplasia returns, the symptoms recur, and the menses diminish or lapse. The breasts apparently store estrin in a mobilizable and active form, and in my opinion they are an indicator of the sufficiency of estrin therapy in all hypo-ovarian states.

The ovarian hormones react differently on the hypertension of these cases. Only progynon-B in large dosage, 50,000 to 100,000 I.U., causes a fall of from 20 to 40 mm. Hg in 24 to 48 hours. It is necessary at first to maintain dosage from 10,000 to 50,000 I.U. 2 to 3 times a week, for months, before the pressure is stabilized at the low level. After the higher dosage of estrin has

produced relief of the symptoms, it is reduced to the individual maintenance requirement. Maintenance dosage is possible with peroral administration of 1200 to 1500 B.U. of dihydroxyestrin, and is sometimes capable of controlling the symptoms in cases diagnosed early. Premenstrually, there frequently occurs a rise in systolic pressure which is soon succeeded by the re-appearance of the migraine and other symptoms; therefore, increased dosage is often required premenstrually to compensate for the normal cyclic rise of estrin values. From 2 to 4 injections of proluton, 1 to 2 I.U., have been required before a fall in systolic pressure has been noted, although it stabilizes the lowered blood pressure for a longer interval.

When x-ray therapy failed to control the convulsions in *case 8*, 1 cc. anterior pituitary injections tri-weekly stopped them, and also reduced the persistent systolic pressure of 155 to 160 mm. Hg to normal. When an enlarged clitoris was observed in May, 1935, pituitary therapy was stopped, and ovarian concentrate, 4 to 8 pills daily, substituted. In 3 months, hair was found daily on the bed linen and in the bath water, and only the abnormal hair could be painlessly depilated. When the systolic pressure fell from 124 to 104 mm., ovarian concentrate therapy was stopped. This time the systolic pressure rose to 128 mm. but after 6 weekly injections of 0.5 to 1 I.U. of prolution it fell to 106 and the diastolic to 72 mm. Hg. Therapy was then discontinued.

Hypertrichosis is apparently controlled in cases receiving either progynon-B, prolution, or the ovarian concentrate, Temporary discontinuance of therapy has usually permitted an increase in hirsutism, a rise in systolic pressure, and recurrence of amenorrhea or hypomenorrhea.

Inadequate estrin therapy has resulted in progression of the symptom state, the plethoric obesity, the hypertension, the hypertrichosis, and a subsidence of menses (fig. 1 *D*). Intensive progynon-B therapy promptly relieved the symptoms.

Ovarian concentrate must be administered for 4 to 6 weeks before its blood pressure-reducing effect is observed, and the systolic pressure is sometimes reduced as much as 45 per cent. Following the administration of 6 to 12 capsules daily, for months, the occurrence of asthenia, dyspnoea, cyanosis of the extremities, and pulmonary edema (in 1 case, which disappeared on stopping therapy), would strongly suggest a toxic effect.

That proluton and ovarian concentrate inhibit the adrenal cortex or the androgenic zone, in the same manner that estrin inhibits anterior pituitary function (26) and causes basophilic castration changes (25), is suggested by the latter findings. This is further supported by the fact that the blood chlorides in *case 1* were reduced from 616 to 486 mg. per cent, by the clinical observation of reduced systolic pressure, loss and depilation of the hypertrichosis, and improved menses in these cases, as well as by results in an unreported group of virilism cases, corresponding to those observed after unilateral adrenalectomy as performed by Broster (22) in the adrenogenital syndrome.

Out of 11 cases (women), clinically diagnosed as Cushing syndrome and

treated by ovarian hormone therapy 6 cases (*1, 2, 3, 8, 10, 11*), who received large doses of progynon-B or DH, alone or combined with proluton in 1 to 2 I.U. over a period of 3 to 12 months exhibited excellent or very good results, *case 4* receiving interrupted therapy for 4 years, and *case 9* for 1 year exhibited consistently excellent results during the period of intensive therapy; *case 6*, receiving low dosage of progynon-DH for 18 months, gradually developed the characteristic features of the syndrome, but subsequent intensive progynon-B therapy promptly relieved the symptoms and the plethora; *cases 7* and *5*, treated for a short period to demonstrate again the effect on hypertension and the symptom complex, I consider untreated. Both these patients died of pneumonia in August, 1936.

The future course of the treated patients cannot be predicted at this time, but progynon-B therapy can be maintained for long periods without untoward effect. We regard this as an endocrine therapy inhibiting anterior pituitary function and basophilic changes. A definite value of the therapy is established by relief from the symptoms and disappearance of clinical and physical manifestations of the benign (but not the malignant) form of the Cushing syndrome. Pregnancy, an acknowledged stimulation of hyperplasia of the pituitary and adrenal glands, immediately preceded the onset of the syndrome in 5 of the 9 married women in this series of 11 cases.

REFERENCES

1. Cushing, H.: Bull. Johns Hopkins Hosp. **50**:137. 1932. J.A.M.A. **99**:281.
2. Erdheim, J.: Beitr. z. path. Anat. u. z. allg. Path. **33**:158. 1903.
3. Rolleston, H. D.: The Endocrine Organs in Health and Disease, Oxford, London, 1936.
4. Leyton, O.: Lancet **1**: 1221. 1934.
5. Kepler, E. J.: Proc. Staff. Meet., Mayo Clin. **8**:102.
6. Hare, D. C., S. M. Ross and A. C. Crooke: Lancet **1**:118. 1935.
7. Lyall, A.: Brit. M. J. **1**:354. 1935.
8. Cahill, G. F.: Surg. Gynec. & Obst. **62**:287. 1936.
9. McCallum, W. G., T. B. Futcher, G. L. Duff and R. Ellsworth: Bull. Johns Hopkins Hosp. **56**:350. 1935.
10. Rasmussen, A. T.: Endocrinology **20**:673. 1936.
11. Fuller, C. J. and D. J. Russell: Lancet **2**:181. 1936.
12. Cohen, H. and J. H. Dible: Brain. **59**:395. 1936. Lancet **1**:455. 1937.
13. Norris, E.: Arrhenoblastoma of Ovary. To be published.
14. Ullom, J. T.: Proc. Coll. Phys. Phila., May 24, 1937.
15. Crooke, A. C.: J. Path. & Bact. **41**:339. 1935. Personal communication, May 1936.
16. Kessel, F. K.: Ergebn. d. inn. Med. u. Kinderh. **50**:620. 1936.
17. Personal communication, May 21, 1937.
18. Cohen, H. and J. H. Dible: Lancet **1**:597. 1937.
19. Grollman, A.: The Adrenals. Williams & Wilkins, Baltimore, 1937.
20. Broster, L. R. and H. W. C. Vines: The Adrenal Cortex, Lewis, London, 1933.
21. Walters, W., R. M. Wilder and E. J. Kepler: Am. J. Surg. **100**:670. 1934.
22. Broster, L. R. and H. W. C. Vines: Brit. M. J. **1**:662. 1937.
23. Crile, G., H. Turner and P. McCullaugh: New York State J. Med. **36**:475. 1936.
24. Lisser, H.: Endocrinology **20**:567. 1936.
25. Nelson, W. O.: Endocrinology **19**:187. 1935.
26. Zondek, B.: Lancet **1**:7. 1936.
27. Dunn, C. W.: Clin. Med. & Surg. **41**:255. 1934.

ASSOCIATION NOTICE

PRELIMINARY ANNOUNCEMENT OF THE ANNUAL MEETING

The twenty-second annual meeting of The Association for the Study of Internal Secretions will be held in San Francisco, California, on June 13th and 14th, 1938. The St. Francis Hotel, Powell and Geary Streets, will be the headquarters for registration and for the scientific and business sessions.

The members of the Local Committee are Dr. C. Frederic Fluhmann, Dr. Frank Hinman, and Dr. H. Lisser, Chairman.

Hotel Accommodation. Reservations for hotel rooms should be made through the Hotel Housing Committee of the American Medical Association (See J.A.M.A., Nov. 20, 1937, page 55). Please address F. C. Warnshuis, M.D., Chairman Hotel and Housing Committee, 450 Sutter St., San Francisco.

PRESENTATION OF PAPERS

Members of the Association and other investigators who wish to present papers will please note the following regulations for the presentation of papers at the coming meetings, especially since they differ in some respects from those of former years.

1. The title of the paper, and a comprehensive abstract *in triplicate,* must reach the President, Dr. F. C. Koch, 947 East 58th St., Chicago, Illinois, not later than March 10, 1938.

2. Non-members who present papers will appear on the program "by invitation." Non-members are therefore requested to have their titles and abstracts introduced by a member.

3. The titles and abstracts of papers submitted for presentation at the meeting will be considered by the program committee and the final program will be announced about April 1, 1938.

4. Papers will be limited to ten minutes for presentation and five minutes for discussion.

5. All papers presented before the Association or read by title are to be submitted, in completed manuscript form, to ENDOCRINOLOGY, either before or immediately after the meeting. It is to be understood, however, that acceptance of a paper for the program does not obligate the Journal to its acceptance for publication.

You are invited to bring this announcement to the attention of your colleagues and associates who have endocrine researches in progress.

CURRENT ENDOCRINE LITERATURE

COLLABORATORS

Fuller Albright
Wayne J. Atwell
Geoffrey Bourne
Israel Bram
John C. Burch
John C. Donaldson
Maurice H. Friedman
Murray B. Gordon
Arthur Grollman
Frank A. Hartman
E. C. Hamblen
F. C. Koch
Edward Larson
J. T. Lewis
Joseph M. Looney
Warren O. Nelson
J. P. Pratt
A. T. Rasmussen
Elmer L. Sevringhaus
L. B. Shpiner
D. L. Thomson
R. L. Zwemer

ADVANCE ABSTRACTS

The potency of certain commercial hormone preparations. D'Amour, F. E., and Marie D'Amour. To appear in this Journal.

A total of 31 commercial hormone preparations, comprising estrogenic, gonadotropic, adrenal cortex and growth substances, were assayed, using the methods employed by the manufacturers. Of these, 8 did not approximate the labelled potency within a reasonable limit. Six others were open to the criticism either that no biologic assay had been performed or that an unusually small unit had been used. The authors suggest that it would be highly desirable for producers of these substances, first, to study the problem of possible deterioration and second, to agree upon standard assay procedures and units to be used.

The inhibition of uterine bleeding with estradiol and progesterone and associated endometrial modification. Hisaw, F. L., and R. O. Greep. To appear in this Journal.

The injection of definite amounts of these crystalline ovarian hormones into castrated monkeys following a standard 'priming' treatment with 100 R.U. estradiol for 20 days permitted a quantitative determination of their effectiveness (given separately or in combination) in the prevention of uterine bleeding and the induction of progestational changes. Amounts of estrin or progesterone, 25 R.U. or 0.25 Rb.U., which given separately did not delay bleeding beyond the time when it would have occurred had no treatment been given, delayed bleeding 8 to 14 days when given simultaneously. Fifty R.U. inhibited bleeding for 9 to 10 days, but when combined with 0.25 Rb.U. or 0.5 Rb.U. the period was extended to 28 and 22 days, at which points the treatment was changed and bleeding had not occurred. Discontinuance of the progesterone precipitated bleeding. Such bleeding, however, was successfully prevented by raising the estrin level to 500 R.U. daily. Three-fourths Rb.U. inhibited bleeding 9 to 19 days, and 1 Rb.U. gave prolonged inhibition (at least 44 days), but bleeding did occur within 4 to 7 days when the dosage was reduced to 0.5 Rb.U. daily. A daily dose of progesterone (0.25 Rb.U.) insufficient to delay bleeding also produced little endometrial modification, but the addition of 25 or 50 R.U. estrin permitted the development of mild progestational changes. One half Rb.U. alone was also ineffective, but in conjunction with estrin produced typical premenstrual endometria. Three-fourths R.b.U. daily produced a fair presecretory condition at 19 days, but after 31 days on 1 Rb.U. the endometrium, in some respects, resembled that of a castrated animal. When progesterone was given at intervals during estrin treatment, it was possible, by adjusting the dosages to convert a premenstrual endometrium back to an estrin and again to a premenstrual condition without the intervention of bleeding. It was concluded from histological studies of the glycogen in the glandular epithelium that both estrin and progesterone favor the deposition of glycogen, but its elimination is dependent upon corpus luteum action.

The role of the hypophysis in cranial osteomyelitis, petrositis and sinus infections. Levene, G., L. F. Johnson, R. M. Lowman, and E. G. Wissing. To appear in this Journal.

In a study originally based on a series of 3000 skull roentgenograms it was found that the architecture of the skull is influenced by the activity of the pituitary gland. The changes are

classified as types 1, 2, 3 and 4. These show variations not only in gross structural appearance, but in vascularity and calcium content. Cranial osteomyelitis of sinus origin and suppuration of the petrous apex occur only in skulls of types 1 or 3. These are associated with overactivity of the anterior lobe of the hypophysis. Sinus infection is also more common in these types.

Gonadotropic potency of the hypophysis in a wild male rodent with annual rut. Wells, L. J. To appear in this Journal.

Hypophyses from 71 male ground squirrels (Citellus) were assayed at approximately monthly intervals during 2 annual sexual cycles. An immature female mouse was given a single hypophysis intramuscularly, killed 72 hours later and weighed. The percentage weight of reproductive organs (ovaries, fallopian tubes and uterus) on body weight for recipients was compared with that of untreated litter mates. In crucial cases, ovaries and uterus were studied microscopically. The vaginal membrane was examined for rupture. Gonadotropic potency of the hypophysis from adult males showed great seasonal variation, being high in males with spermatozoa and extremely low in adults sacrificed during the long period of aspermia and regressed secondary reproductive organs. This variation runs parallel to the seasonal cytological fluctuations within the hypophysis which have been previously reported, and also to the seasonal variation within male reproductive organs. The fact that the hypophysis showed greatly diminished potency during the long aspermatic period, rather than an absence of potency, supports the previous contention that male reproductive organs are not entirely inactive during the non-rutting period. Seasonal reduction of gonadotropic potency was prevented, or at least inhibited, both by bilateral castration and by subjection of breeding adults to continuous and constant low temperature. Potency of the hypophysis from prepuberal males was either slight or absent at 2 months of age, low at 3-5 months, and high several weeks before spermatozoa expectancy.

ADRENALS

Comparison of the weights of suprarenals of dogs in oestrus, pregnancy and lactation. Baker, D. D., J. Morphol. **62**:3. 1938.

Comparisons have been made of the weights of the suprarenals of 525 mature female dogs in dioestrus, estrus, pregnancy and lactation. Tables and graphs show that there was a slight increase in the size of the suprarenals during estrus. There was hypertrophy of both the cortex and the medulla in the estrous dogs. Only slight increase was shown in the weights of the glands of pregnant animals when the net body weight was considered and no increase when the total body weight was used in the study. There was no hypertrophy of the glands of lactating animals. There was only a slight variation in the seasonal occurrence of estrus. From 34 to 37% of the total numbers of mature females examined in the seasons were found to be in estrus. From 13 to 16% of the total numbers were found to be pregnant.—Author's Abst., Wistar Bib. Service.

The metabolism of adrenalectomized rats. Carr, C. J., and F. F. Beck, Am. J. Physiol. **119**: 589. 1937.

The metabolism of 10 white rats was determined by a modified Haldane open circuit apparatus before and after bilateral adrenalectomy. Following the operation, there was a drop of 25% in the total metabolism. Eight of the 10 animals showed a rise in metabolism preceding death. Normal animals on a restricted diet showed no such changes in metabolism.—F. A. Hitchcock.

Action of gonadotropic hormone on the adrenal cortex (L'action de l'hormone gonadotrope sur la corticale surrénale). Crainiceano, A., L. Copelman, E. Banu and Sarbou, Compt. rend. Soc. de biol. **125**:227. 1937.

Gonadotropic hormone administered to 20 mature male rats caused disappearance of the lipoidal vacuoles in the fascicular zone of the adrenal cortex, resulting in the formation of a compact zone containing small spongiocytes. These adrenals weighed 70-120 mg. as compared with the normal 50-60 mg. It was suggested that the principle in the urine of cancerous subjects which has been shown to cause similar fascicular zone modifications is a gonadotropic substance.—E. Cutuly.

Structural and functional reconstitution of ultracentrifuged rat adrenal cells in autoplastic grafts. Dornfeld, E. J., Anat. Rec. **69**:229. 1937.

Stratification and displacement of cellular components was produced in rat adrenal cells

by 30 minutes of ultracentrifuging at 400,000 times gravity. After 3 hours in isotonic salt solution almost complete recovery of the nuclear elements occurs. Glands centrifuged and halved were autoplastically grafted into the abdominal muscles of 46 rats. Typical host-transplant reactions result, and vascularization sets in on the third day. Rapid reconstitution and regeneration of cortical cells occurs, during which the hitherto shrunken Golgi mass expands and small lipoidal droplets appear in the cytoplasm. The fully regenerated grafts show normal histological features, save for the frequent occurrence of tissue spaces filled with extravasated blood and secretion products. Medullary cells do not regenerate, but this phenomenon is independent of the effects of centrifuging. Normal functional capacity is indicated by the cytological changes; by the fact that implanted animals without accessory tissue outlive adrenalectomized controls; that removal of the grafts is followed by insufficiency symptoms and death; and that animals with implants show normal weight gains, whereas completely adrenalectomized ones do not. It is concluded that morphologic displacement of the cellular components has no necessarily deleterious effect on these components or on the viability and functional capacity of the cells. The use of centrifuging methods for the determination of relative weights of cell parts may therefore be considered valid.—Author's Abst., Wistar Bib Service.

Rôle of the adrenal cortex in the production and utilization of vitamin C. Its influence on the structure of the teeth in the albino rat. Perla, D., and M. Sandberg, Arch. Path. **23:**372. 1937.

In higher animals, vitamin C is present practically in all tissues, but it is highest in the glandular tissue, and lowest in muscle. As the adrenal glands are tissues of high metabolic activity, it utilizes large amounts of reducing substance as cevitamic acid. It is suggested that vitamin C forms an oxidation reduction system with glutathione. Adrenalectomy in the rat results in microscopic changes in the teeth consisting of the disappearance of the predentin zone, or in condensation or calcification of this zone and in the irregularity of the odontoblast layer, with vacuolization and pyknosis of the nuclei. Occasionally there occurs metaplasia of the dentine with the formation of islands of osteoid tissue. These changes were not observed in rats receiving daily injections of methyl cyanide.—L. B. S.

Maintenance of life in adrenalectomized animals with flavin phosphate. Pijoan, M., and S. A. Oberg, Proc. Soc. Exper. Biol. & Med. **36:**187. 1937.

The administration of 0.15 cc. of flavin phosphate daily to adrenalectomized rats permitted normal activity and growth during the course of the experiment (3 weeks), whereas similar animals receiving 0.03 cc. daily died from 10 days (75%) to 3 weeks. Animals given the same dose of flavin phosphate, plus 50 mg. of ascorbic acid daily, survived 3 weeks, but lost weight and then died during the fourth week. Adrenalectomized rats receiving 0.25 cc. of cortin daily fared well on an ordinary diet, but died on a flavin-free diet. Untreated adrenalectomized controls died in 4 days. It is suggested that ascorbic acid acts synergistically with flavin phosphate to maintain life after adrenalectomy, and that cortin is unable to maintain life in animals on a flavin-free diet.—M. H. F.

Epinephrine output from the adrenal glands in experimental diabetes. Rogoff, J. M., and E. Nola Nixon, Am. J. Physiol. **120:** 440. 1937.

Additional evidence is presented showing that the development, severity and course of experimental pancreatic diabetes are not influenced by reduction or suppression of epinephrine secretion from the adrenals. Diabetic animals, not subjected to surgical interference with the adrenals, sooner or later develop reduced or suppressed secretion of epinephrine. This appears to be due primarily to the diabetic state. Electrical stimulation of the splanchnic nerve is capable of increasing the epinephrine output up to or above the normal level, in those animals that show a marked reduction in output.—Authors' Abst.

A sensitive method for quantitative estimation of epinephrine in blood. J. M. Rogoff, Proc. Soc. Exper. Biol. & Med. **36:** 441. 1937.

After unilateral extirpation of the superior cervical ganglion, repeated intravenous injections of small quantities of epinephrine resulted in a progressive increase in sensitivity to the point where subsequent intra-arterial injections of still small quantities could be detected by the reaction of the denervated eye structures. In such fashion it was possible to detect the epinephrin in dilutions of 1:200,000,000.—M. H. F.

Is the transmission of the nerve impulses to the cat's uterus electrical or chemical? Ross, J. F., Am. J. Physiol. **119**:527. 1937.

Advantage was taken of the observation that, while piperidinomethylbenzodioxane (933F) has little value in diminishing the responses of various organs to nervous excitation, it markedly decreases the response to adrenalin or to sympathin. This was applied to a study of the possible differential effect, which may occur in the response of the cat's uterus to nervous excitation and to adrenalin. In suitably prepared animals (pregnant and non-pregnant), nervous stimulation (hypogastric nerve) and adrenalin administration was recorded, i.e., isotonic contraction of the uterine muscle; this was followed by intravenous administration of piperidinomethylbenzodioxane in doses of 3-10 mg. per kg. In non-pregnant animals, typical relaxation followed hypogastric stimulation, sometimes preceded by a preliminary contraction. Adrenalin caused only relaxation. Following 933F administration, nerve stimulation caused a relaxation only slightly smaller than before, but the response to adrenalin was very markedly decreased. In pregnant animals hypogastric stimulation or adrenalin injection induced uterine contraction. Administration of 933F had no effect upon the response to nervous excitation, whereas the contractions resulting from adrenalin were markedly decreased. Monnier and Bacq postulated the existence of both electrical and chemical transmission of nerve impulses to smooth muscle, on the basis of their work with the nictitating membrane. Piperidinomethylbenzodioxane exerts a differential effect upon the action of nerve stimulation and of adrenalin in a manner identical with that produced by them in the nictitating membrane, but it is believed this evidence is contrary to Monnier and Bacq's assumption, since they felt that chemical transmission alone obtained in muscles responsive only to repeated nerve volleys, and showing no spike action potentials, (e.g., pregnant cat uterus). The writer believes rather that, while 933F may increase the polarization of the cell membrane, rendering it less permeable to adrenin, sympathin liberated intracellularly as a result of nerve stimulation would be unaffected. Hence, it is unnecessary to postulate both electrical and chemical transmission of nerve impulses to smooth muscles.—H. O. Haterius.

A comparison of the metabolic effects of subcutaneous and intravenous epinephrine injections in normal and hypophysectomized rats. Russell, Jane A., and Gerty T. Cori, Am. J. Physiol. **119**: 167. 1937.

Approximately 40 normal and 40 hypophysectomized animals, 150-200 gm. in wt., were used in this comparison. Hypophysectomized animals were used 10 days-four months after operation. All experiments were carried out under amytal or nembutal anesthetic. The effect of epinephrine on muscle glycogen, hexosemonophosphate, blood sugar and intravenous glucose tolerance of anesthetized normal and hypophysectomized rats was determined. When the epinephrine was given subcutaneously, all of these effects were less in the hypophysectomized than in the normal rats; but when the epinephrine was given in a slow intravenous infusion, the effects of hypophysectomized and normal animals were similar. The differences in reaction of the normal and operated rats to subcutaneous injection of epinephrine were therefore attributed to a diminished rate of absorption of the epinephrine from the subcutaneous tissues in the absence of the hypophysis. The operated rats showed a lower tolerance for intravenous glucose than did normals, the reduction being about the same order of magnitude as the decrease in metabolic rate. The operated rats exhibited an abnormally high renal threshold for glucose and, whether epinephrine was given or not, excreted less glucose than did the normals. —K. A. Brownell.

The effect of regeneration of the nerve supply on the sensitivity of the denervated nictitating membrane to adrenine. Simeone, F. A., Am. J. Physiol. **120**: 466. 1937.

The development of the sensitivity of the cat's nictitating membrane to adrenine after denervation is a gradual process increasing rapidly at first and then more slowly until a maximum is reached after about 2 weeks. The sensitivity is greater after postganglionic than preganglionic denervation. With regeneration of the nerve supply the increased sensitivity began to decline, becoming normal for preganglionic in an average of 2 weeks and nearly normal for postganglionic in an average of 7 weeks. Regeneration was somewhat faster after crushing than after cutting the nerves. There is a discussion of the nature of sensitization.—F. A. H.

Urinary excretory rate of adrenalectomized rats. Beaird, R. D., Jr., and H. G. Swann, Proc. Soc. Exper. Biol. & Med. **36**: 194. 1937.

The daily urinary excretion was measured in untreated rats, and rats after adrenalectomy. The daily output of the latter was lower than normal if untreated, but 5 times as great as the normal output when the rats were being maintained by salt therapy. When this therapy

was removed and the animals replaced on an ordinary diet, the urinary volume decreased to the normal level in 24 hours, and the rats soon succumbed. The therapeutic dietary régime did not affect the daily output of normal rats.—M. H. F.

The influence of adrenal cortical hormone upon electrolyte and fluid distribution in adrenalectomized dogs maintained on a sodium and chloride free diet. Swingle, W. W., W. M. Parkins, A. R. Taylor and H. W. Hays, Am. J. Physiol. **119**:684. 1937.

Two normal adult male dogs on a sodium and chloride free diet remained in good health over an observation period of 15 days. The blood electrolyte pattern remained normal, but some hemoconcentration occurred. Studies of the blood electrolytes and urine electrolyte excretion were made on 2 bilaterally adrenalectomized dogs maintained on adrenal cortical extract, and fed on a salt-free diet. The blood electrolyte pattern remained normal, but definite evidence of hemoconcentration was observed when the extract dosage was somewhat decreased. On reducing the extract still further, the animals went into insufficiency. During this period there was complete suppression of urine excretion. The blood electrolyte concentration showed only a small decrease in Na and Cl and slight increase in K. However, despite this fact the animals showed all the signs of insufficiency. Marked hemoconcentration was noted. On resumption of extract, marked blood dilution was noted, and after a few hours the electrolyte pattern was within normal range, despite the fact no water or salt had been administered except the negligible amount contained in the extract solution. Under the conditions of these experiments, severe insufficiency can occur with hemoconcentration, but the changes in Na, K and Cl concentration are insignificant.—Lena Lewis.

A study of water intoxication in the intact and adrenalectomized dog and the influence of adrenal cortical hormone upon fluid and electrolyte distribution. Swingle, W. W., W. N. Parkins, A. R. Taylor and H. W. Hays, Am. J. Physiol. **119**:557. 1937.

The effect of water intoxication, produced by giving 25 cc. water per kg. body weight hourly, was studied in normal and adrenalectomized dogs. The water was given until convulsions resulted. Four intact dogs were studied. Serum Na and Cl values were low, and K changes were insignificant during period of water intoxication. Normal dogs usually recovered spontaneously after withdrawal of water. Adrenalectomized dogs maintained by cortical hormone showed hemoconcentration, and reduced Na and Cl when water intoxication developed. They never recovered spontaneously, but could be restored by intravenous salt-free cortical hormone or intravenous hypertonic sodium chloride. The dog restored with cortical hormone had very low serum Na and Cl concentrations. Priming the dog immediately before giving the water, with either intravenous hypertonic sodium chloride or cortical hormone was effective in warding off symptoms of intoxication.—Lena Lewis.

The effects of epinephrine on urine excretion in dogs. Toth, L. A., Am. J. Physiol. **119**:140. 1937.

The effects of epinephrine infusions on the anaesthetized dog were studied. The rates of infusion (intravenously) varied from 1.6 to 7.72 per kg. body weight per min. Rates of 3.02 or less produced a polyuria, and above 5.62 a definite oliguria. However no absolute threshold value was found. Intramuscular and subcutaneous injections of epinephrine produced polyuria. The urea and chloride excretions varied directly with the volume of urine. Proteinuria usually accompanied the oliguria. The effects produced by the epinephrine infusions were similar to these produced by anoxia.—Lean Lewis.

Effect of estrus (pseudopregnancy) and certain pituitary hormones on the life-span of adrenalectomized animals. Swingle, W. W., W. N. Parkins, A. R. Taylor, H. W. Hays and J. A. Morrell, Am. J. Physiol. **119**:675. 1937.

During the period of pseudopregnancy following estrus, the adrenalectomized bitch remains in good condition without cortical extract. Near the end of the pseudopregnant period, hemoconcentration occurs and blood pressure falls. Extract of menopause urine effectively induces estrus. Attempts to prolong the life-span of adrenalectomized bitches (not in heat) by injections of large amounts of various ovarian and pituitary hormones were not successful. APE prolongs the life-span of bilaterally adrenalectomized cats. The sex of the animal, or the presence or absence of sex glands has no apparent effect on the action of the extract. Large daily injections of the crystalline hormone of the corpus luteum do not prolong the life-span of bilaterally adrenalectomized cats.—Author's Summary.

Relation of carbohydrate to salt and water disturbances after adrenalectomy (Der Zusammenhang von Kohlenhydratstoff wechselstörung mit Natrium- und Wasserstoffwechsel beim nebennierenlosen Tiere). Verzar, F., and L. Laszt, Verh. Schweiz. Physiol. 11: 19. 1937. Physiol. Absts. **22:** 413.

Administration of glucose solutions to the adrenalectomized rat is fatal because, owing to the failure of glucose absorption, water and Na pass into the intestine in large quantities to maintain osmotic equilibrium, so that the rat dies of Na deprivation. Na content of blood sinks from 300 to 234 mg.% when the experiment is made 6 days after removal of the adrenals. Glucose plus NaCl and Na_2CO_3 is not lethal. This may explain the old observation of the apparently favorable influence of Na on adrenal deprivation. It is suggested that the root cause of all metabolic disturbances following the loss of the adrenals is paralysis of the flavin respiratory enzyme, which is broken down, as the flavin in the tissues becomes diffusible.

Adrenal cortex and transport of fat (Nebennierenrinde und Fettwanderung). Verzar, F., and L. Laszt, Biochem. Ztschr. **288:**356. 1936.

The authors had shown previously that phosphorus poisoning (5 days) did not produce fatty degeneration of the liver in adrenalectomized rats. In the present experiments it was shown that degeneration of the liver (increase of fat of 100%) was produced under the same conditions, if cortical extract was given. Before the operation, the animals were fed on a diet rich in fat, and afterwards on a normal diet. It is concluded that the cortical hormone is not only necessary for fat resorption, but also for the mobilization of fat depots. In 2 experiments, flavin phosphoric acid had an effect like cortical extract on the degeneration of the liver. It was much smaller, however.—E. Bozler.

Significance of increased frequency of selective cortical necrosis of adrenal as cause of Addison's disease. Wells, H. G., Eleanor M. Humphreys and Emma G. Work, J.A.M.A. **109:**490. 1937.

The authors state that the increasing frequency of contacts with new chemicals, whether in industries or in therapeutics, is bringing about new disease conditions. They suspect that an important disease condition, namely, 'suprarenal cortex atrophy' leading to Addison's disease, may be produced by a new therapeutic chemical agent, 'germanin.' Of the last 9 cases examined by the authors 6 were of the 'atrophy' type, better designated as 'selective destruction of the adrenal cortex.' Since that time, 3 of 7 additional cases of Addison's disease observed were associated with cortical atrophy, and 4 with tuberculosis. The authors describe animal experiments on the action of germanin injections. These experiments demonstrated a marked, and perhaps even a special, susceptibility of the adrenal cortex. It is improbable that such a rarely used drug as germanin is responsible for many cases of Addison's disease. However, in a study of the toxic potencies of drugs, the adrenal glands are rarely considered. It would seem probable that therapeutic chemicals in more common use than germanin may be found to have a special injurious effect on the adrenal cortex, especially under conditions of unusual susceptibility.—Abst., A. M. A. Service (Condensed).

Extracts of suprarenal cortex in treatment of Addison's disease. Wilkinson, J. F., Lancet. **233:**61. 1937.

In this study of 9 cases, the following characteristic features of Addison's disease were utilized: asthenia and muscular weakness, digestive disturbances, abnormal pigmentation, low weight, low blood pressure, subnormal temperatures, and changes in blood chemistry. Of these selected cases 7 were married women, aged between 32 and 56 years, and 2 were males aged 17 and 35 respectively. Intravenous and intramuscular injections were used, with daily doses varying from 25 to 50 cc. Four patients died in spite of the intensive treatment, and 5 patients responded to this therapy, the longest period of survival being 3 years and 1 month, and the shortest 19 months. The use of the extracts relieved the asthenia, muscular weakness, and the digestive disorders; the appetite returned, weight increased, pigmentation faded, and there was slight improvement in the subnormal temperature. The blood pressure may take 2 to 3 months to show significant improvement, and then only if the extract is given in conjunction with NaCl therapy. High blood urea returned to normal, the NaCl, and PO_4 also returned to normal values under this form of therapy. However, it was found that the cortical extract had no effect on the slight hypochromatic microcytic anemia, and the B.M.R. was unaltered.—L. B. S.

The rôle of the adrenal gland in blood sugar recovery after insulin hypoglycemia. Zucker, T. F., and B. N. Berg, Am. J. Physiol. **119**: 539. 1937.

The purpose of the work was to observe the response to insulin after medullectomy, after bilateral adrenalectomy in acute experiments, and after bilateral adrenalectomy in survival experiments. Both dogs and cats were used, and insulin in amounts from 0.1 to 2.0 U of Lily's clinical preparation was administered. Blood sugar was followed by the Schaffer-Hartman method. All operations were performed under nembutal anesthetic, and medullectomy was carried out according to the curette method of Houssay. It was found that the adrenal medulla was not essential to blood sugar recovery after hypoglycemia. Bilateral removal of the medulla without further interference with normal function abolished the 'sympathico-adrenal emergency mechanism' at hypoglycemic levels. That absence of both adrenals does not prevent recovery but might delay it was shown by both acute and survival experiments.—K. A. Brownell.

ENDOCRINE GENERAL

Paroxysmal hypertension and other clinical manifestations associated with benign chromaffin cell tumors (phaeochromocytomata). Howard, J. E., and W. H. Barker, Bull. Johns Hopkins Hosp. **61**:371. 1937.

The authors have carefully analysed the clinical picture presented by 18 proven cases of phaeochromocytomata with paroxysmal hypertension. The analysis showed that these tumors vary widely in size and in their sites of location in the abdominal cavity. They are by no means all detectable by physical examination, plain x-ray plates or pyelographic studies. Surgical removal of the offending tumors has been uniformly successful in effecting cures in the cases surviving operation. The clinical picture during paroxysmal episodes forms the chief basis on which diagnosis may be established. Among the more useful and significant symptoms accompanying the paroxysms of hypertension are the following: blanched or mottled cold extremities, palpitation, nausea, sweating, headache and precordial pain or signs of sudden cardiac incompetence. In general the clinical manifestations resemble those of sudden large discharges of epinephrine into the circulation.—A. G.

Endocrine therapy in chronic cystic mastitis. Lewis, D., and C. F. Geschickter, J.A.M.A. **109**:1894. 1937.

The authors maintain that the treatment of adenosis or of cystic disease by excision of the nodule-containing tissue is unsatisfactory, since the condition is chronic and nearly always bilateral, and similar tumors tend to appear in the same or in the opposite breast following excision. However, such local excision should always be performed in order to permit microscopic study if the clinician is unable to rule out carcinoma. If the painful nodular tissue is allowed to persist without further treatment after its benign nature is clinically established, the patient often continues to be apprehensive of cancer despite reassurance by the physician. As the result of changing physicians, multiple excision and amputation of one or more breasts is the rule in at least one-third of such cases. The authors believe that the performance of single or bilateral mastectomies is not indicated. A review of the data on file in the Surgical Pathologic Laboratory has convinced them of the benign nature of these conditions and the absence of a relationship to cancer. Of 1,048 cases of adenosis and cystic disease, 523 have been followed for more than five years, and carcinoma of the breast developed in only four patients. In carrying out the endocrine treatment in these cases, it must be borne in mind that in general the various forms of chronic cystic mastitis are self limited and tend ultimately to regress. The hormone therapy is a convenient form of palliative treatment preventing needless mutilating operations and, if it is properly used, it speeds the regression of the disease. The results are most satisfactory in persistent painful breasts and in early adenosis. Successful endocrine therapy in chronic mastitis requires relatively high doses of estrogen. The estrogen is never given during menstruation, and treatment is usually continued for a period of six months. In some cases in this series estrogen plus pituitary lactogenic substance was tried instead of estrogen alone.—Abst., A. M. A. Service.

Cardiovascular properties of epiphyseal extracts (Sur les propriétés cardio-vasculaires des extraits épiphysaires). Malméjac, J., and E. Desanti, Compt. rend. Soc. de biol. **125**:475. 1937.

Aqueous extracts of beef pineal glands injected intravenously into dogs caused tachycardia and arterial hypotension.—E. Cutuly.

GONADS

Ovogenesis during sexual maturity. The first stage, mitosis in the germinal epithelium, as shown by the colchicine technique. Allen, E., and R. N. Creadick, Anat. Rec. **69**:191. 1937.

Active mitosis in the germinal epithelium, the first stage of ovogenesis, occurs in the ovaries of the mature mouse at estrus. The evidence, accentuated by arrest of mitosis with colchicine, firmly establishes this fundamental process in this mammal.—Authors' Abst., Wistar Bib. Service.

Development of the mammary gland of the rat. A study of normal, experimental and pathologic changes and their endocrine relationships. Astwood, E. B., C. F. Geschickter and E. O. Rausch, Am. J. Anat. **61**:373. 1937.

The development of the mammary gland during the first 6 weeks of life is independent of the gonad in both sexes. At puberty the female gland rapidly increases in size by extension of the duct tree and this effect can be obtained in castrates with small doses of estrone. The cyclic changes concurrent with the estrous cycles are reproducible in castrates by the alternate administration of estrone and pituitary lactogenic substance. Lobule formation in the female occurs only with pregnancy and can be induced experimentally with gonadotropic substances, but not with estrone or progesterone (progesterone has no demonstrable effect on the rat mammary gland). The mammary gland of the male at puberty develops lobules of acini; this type of growth can be induced by gonadotropic substances in the immature intact male or by testosterone in castrates. Following hypophysectomy the mammary gland will not respond to estrone but regresses; the effects of hypophysectomy are reproducible in intact animals by partial starvation. Large doses of estrone interfere with normal development and result in a stunted abnormal gland. With further dosage such a gland shows secretory changes, marked cyst formation, premature ageing with an increase in fibrous stroma, and irregular ducts with epithelial proliferation. The cystic changes are not specific for estrin as similar abnormalities result from excessive gonad stimulation in the male and from large doses of testosterone.—Authors' Abst., Wistar Bib. Service.

Metabolic changes in liver, kidney and anterior hypophysis of pregnant, parturient and lactating rats. Andersen, Dorothy H., Margaret R. Prest and J. Victor, Am. J. Physiol. **119**:445. 1937.

The metabolism of rat liver, kidney and anterior pituitary was studied in vitro at various stages in the reproductive cycle. The most pronounced changes occurred in the anterior pituitary. Expressed in cm./gm./min. its oxygen consumption was as follows: parturition 20.8, lactation 15.3, pro-estrus 15.5, estrus 13.3, pregnancy 12.8, di-estrus 11.3, and spayed 10.8. The changes in metabolism were associated with changes in gland weight, but not with cytological changes. The metabolism of the liver was highest in pregnancy, while the metabolism of the kidney was depressed in pregnancy and at parturition, but normal in lactation. The R.Q. of the liver was elevated in pregnancy and in lactation.—F. A. Hitchcock.

Theelin therapy in psychoses: effect in involutional melancholia and as an adjuvant in other mental disorders. Ault, C. C., E. F. Hoctor and A. A. Werner, J.A.M.A. **109**: 1786. 1937.

For all practical purposes theelin seems to be specific in involutional melancholia, the apparent recovery rate being 92% in the series treated by the authors. Massive doses of from 30,000 to 40,000 I.U. for the first month of treatment accelerate the recovery rate in involutional melancholia, the hospitalization being reduced to an average period of 3 months. Theelin is indicated for any woman during the climacteric having disturbing mental aberrations, whether mild or severe. Theelin therapy is efficacious in relieving distressing symptoms of the climacteric in other types of psychoses, many patients being improved to the extent of recovery.—Abst., A. M. A. Service.

Treatment of puerperal bleeding with estrin (Die Behandlung von Wochenbettsblutungen mit Follikelhormon). Bauer, W., Zentralbl. f. Gynäk. **61**:2693. 1937.

Seven cases are selected, from the group treated for bleeding in the latter part of the puerperium, to illustrate the successful management with estrin therapy. The method is not proposed when any of the products of conception are retained. It is based on the theory that

the bleeding is analogous to menorrhagia associated with underactive ovaries in the non-pregnant woman. Doses of 10,000 M.U. seemed to be best, repeated at 2 to 3-day intervals, and results were secured in a few days. Failure of the therapy does not prove that the bleeding is due to retained placenta.—E. L. S.

Conclusions about the value of the chemical test for pregnancy of Visscher-Bowman (Ein Schlussurteil ueber den Wert der chemischen Schwangerschaftsreaktion nach Visscher-Bowman). Belonoschkin, B., Zentralbl. f. Gynäk. **61**:2797. 1937.

Based on 100 cases and literature review of published results, it is concluded that the accuracy of the test is so low as to make it undependable; that the test is frequently positive is probably due to coincidental reaction.—E. L. S.

Inhibiting action of testosterone on the plumage of a Sebright capon (Action inhibitrice de la testostérone sur le plumage d'un coq Sebright castré). Champy, C., Compt. rend. Soc. de biol. **125**:329. 1937.

Injection of testosterone sufficient to cause restoration of the comb in a Sebright capon had the same effects on plumage as small doses of folliculin: enlargement of the dorsal and wing feathers and disappearance of smooth barbules.—E. Cutuly.

The hormone of the corpus luteum. Corner, G. W., Edinburgh M.J. **44:** 1937.

This is an account of the development of our present knowledge of the subject.—J. C. D.

Relations between the male and female hormones. Quantitative study of the antagonism between testosterone and folliculin (Sur les rapports des hormones male et femelle. Testostérone et folliculine. Etude de leur antagonisme). Courrier, R., and G. Cohen-Solal, Compt. rend. Soc. de biol. **124**:925. 1937.

Dihydrofolliculin in doses of 0.02 mg. of the crystalline substance when injected into spayed white rats produced estrous changes in all. Testosterone acetate in doses of from 5 to 30 mg. per day failed to give any signs of estrus in 12 spayed rats. To a dose of 0.02 gm. of folliculin, testosterone was added in amounts varying from 5 to 20 mg. Twenty control and 54 experimental animals were used. With 5 mg. of testosterone, 6 of 12 animals failed to show estrous changes. The inhibitory effect of the testosterone increased with the dose until with 20 mg. estrus was entirely prevented. Testosterone acetate in this dosage, therefore, does not have an estrogenic effect, but is actually antagonistic to folliculin.—J. C. D.

Preliminary communication on the estrogenic activity of polymerized anol (Osservazioni preliminari sulla attività estrogena dell'anolo polimerizzato). Dansi, A., E. Morelli and A. Sempronj, Biochim. e. terap. sper. **24**:282. 1937.

Anol (p-oxypropenylbenzene) is polymerized on standing. The resulting substance shows high estrogenic potency as shown by the Allen-Doisy test.—A. E. Meyer.

Experimental study of anti-gonadotropic sera (Contribution à l'étude expérimentale des sérums antigonadotropes). Demanche, R., G. Laroche and H. Simonnet, Compt. rend. Soc. de biol. **125**:112. 1937.

The increase in weight of the seminal vescicles in mice (14-16.5 gm.) produced by 2 M.U. of gonadotropic extract alone was prevented by simultaneous administration of 1 cc. of serum from rabbits which had been injected twice a week for 3 weeks with 1800 M.U. of the extract. Serological tests indicated that the anti-gonadotropic power of such serum was not necessarily accompanied by the property of fixing complement in the presence of the extract used as the antigen.—E. Cutuly.

Hormone therapy for insufficient lactation in women (Hormonale therapie der ungenügenden Laktation bei der Frau). Effkemann, F., Zentralbl. f. Gynäk. **61**:2686. 1937.

In about 30 women with evidence of small breasts and little breast growth during pregnancy, previous experience showed that milk secretion was inadequate. During the 9th month they were given 50,000 I.U. estrin every third day, and during the 10th month 10,000 I.U. of estrin plus 5 mg. progestin at the same intervals. The use of this type of therapy for only the last month gave less help than the complete program, which was conspicuously successful. Progestin alone accomplished no definite results. Prolan therapy was likewise without demonstrable benefit.—E. L. S.

The inhibitory factor of the corpus luteum hormone in the genesis of epithelial metaplasia (Der Hemmungsfaktor des Corpus-luteum-Hormons in der Genese der Epithelmetaplasien). Effkemann, G., and L. Herold, Zentralbl. f. Gynäk. **61**:2865. 1937.

Using intact rats and doses of 200 to 1000 I.U. of estrin, there was either pretreatment or simultaneous treatment with 1 to 2 mg. progestin. Treatments were carried as far as 2 to 3 months. Progestin prevented or eliminated the metaplastic changes in epithelium of the cervix, confirming earlier work of others.—E. L. S.

Growth and basal metabolism. VI. Changes in the basal metabolism in children during puberty. Nakagawa, I., Am. J. Dis. Child. **53**:991. 1937.

The basal metabolic rate, the body height and weight of 24 Japanese children (10 boys and 14 girls) was studied every 6 months throughout the pubertal period. At puberty there was a higher level and an increase in total heat production, weight and height. This increase began at the age of 11 or 12 years in the boys, and at 10 or 11 years in the girls, reaching its maximum at the age of 12 or 13 years in the boys and at 11 or 12 years in the girls, and ending at the age of 15 or 16 years in boys and 14 or 15 years in girls. The maximum was reached before sexual maturity was established in boys and catamenia in girls. This increase in total heat production probably occurs in preparation for the physical changes which take place at puberty.—M. B. G.

Effects of androgenic substances in the female rat. Nelson, W. O., and G. Merckel, Proc. Soc. Exper. Biol. & Med. **36**:822. 1937.

The daily injection of 2.0 mg. of testosterone, androstane-dione, androstene-dione, inhibited the estrous cycle in female rats. The injection of cisandrostene-diol, or dehydroandrosterone, resulted in markedly prolonged periods of estrus. Similar results were obtained by the injection of these latter compounds into hypophysectomized females, but not in spayed females.—M. H. F.

The nomenclature and standardization of the female gonad hormones (Die Benennung und Wertbezeichnung der weiblichen Keimdrüsenhormone). Nürnberger, L., Zentrlbl. f. Gynäk, **61**:2258. 1937.

This is a review of the structural formulae, simpler chemical properties, nomenclature, and methods for bio-assay of the estrogenic and progestational hormones to date.—E. L. S.

The effect of a lysine deficient diet on the estrous cycle. Pearson, P. B., Am. J. Physiol. **118**:786. 1937.

In view of the report that lysine is necessary to ovulation (Courrier and Raynaud), 6 groups of rats were fed on different vitamin-adequate diets, with gliadin as the chief protein source. Rats fed upon diets containing 10% gliadin displayed within 2 weeks irregular cycles or complete cessation of cyclic activity, accompanied by an initial weight decrement. Transfer to a diet containing 18% gliadin was followed by a slight weight increase. Reduction of gliadin content by 3.4%, with addition of a hexone preparation, resulted in a marked increase in growth rate with a concurrent resumption of cyclic activity. Lysine appeared to be the limiting factor rather than inadequate nutrition, since a series on an 18% lysine diet showed fair growth rate increment but no cyclic activity. The addition of d-lysine dihydrochloride to the diet, however, resulted in marked increase in rate of growth and normal estrual cycles. The author believes the demands for growth are satisfied before those incident to the cycle and, further, that lysine may apparently be a specific factor in the estrual project.—H. O. Haterius.

Transmission of estrogenic substances from animal to animal. Perry, Isabella H., and A. Palmer, Proc. Soc. Exper. Biol. & Med. **36**:122. 1937.

Untreated castrate mice came into estrus when placed in a cage with mice whose skin had been painted with 200 I.U. of estrone, or placed beneath the wire floor of the painted animals so as to come in contact with the excreta. Since muzzled mice in both groups of untreated animals also came into estrus, it is concluded that the source of the estrin contamination was from both the skin and excreta, and through both skin and mouth.—M. H. F.

The inhibition of oestrous cycles in the albino rat by progesterone. Phillips, W. C., Am. J. Physiol. **119**:623. 1937.

Previous reports of estrual inhibition by various corpus luteum preparations have lacked the conclusive proof that the hormone itself was the responsible factor, especially in view of the fact that various non-specific substances also inhibited cyclic activity. Isolation of the

corpus luteum hormone in pure form, however, has made possible the testing in such a way as to obviate the possibility of toxic or non-specific effects. Two preparations, alpha-progesterone (Allen and Goetsch) and synthetic alpha-progesterone (Merck) were used in this study, in which it was found that crystalline progesterone, in subcutaneous doses of 1.5 mg. daily or greater, inhibited estrous cycles in the normal female. Smaller doses were ineffective. Cycles were resumed within 3 to 5 days following the last injection. Adequate injections made 3 days prior to an expected estrus inhibited that period of heat; if instituted later than the third day, however, the hormone was ineffective in inhibiting the next expected estrus. In view of the relatively large doses required (1.5 to 4 mg. per day) to inhibit cyclic activity, the author feels it improbable that under physiological conditions progesterone in itself is responsible for the normal inhibition of estrus and of ovulation obtaining during pregnancy in the rat.—H. O. Haterius.

Ascorbic acid during pregnancy in the rabbit. Pincus, G., and J. Berkman, Am. J. Physiol. **119**:455. 1937.

Measurements were made of the ascorbic acid concentration in the corpora lutea, the rest of the ovary, the uterus and the urine at various stages of pregnancy from the 1st to the 28th day. Two essential observations resulted: *1.)* correlation of uterine ascorbic acid concentration with specific pregnancy stages, and *2.)* a correlation between ascorbic acid concentration and corpus luteum growth. Appropriate tissues were removed, weighed and extracted. Determinations were made by titration with 2 to 6 dichlorophenol-indophenol standardized with crystalline ascorbic acid, the extraction fluid consisting of 2 parts 8% trichloracetic acid, 1 part metaphosphoric acid and 1 part distilled water. Corpora lutea showed a maximum ascorbic acid concentration on the third day. Non-luteal ovarian tissue also showed an increase during the first 6 days. Changes in growth rate lagged behind changes in ascorbic acid concentration except in late pregnancy, which implied that increase and decrease in ascorbic acid concentration are followed by corresponding changes in the rate of corpus luteum growth. In the uterus, a small increase in ascorbic acid occurred during the first 6 days of pregnancy, with a slight decline from the 9th day on. Urinary ascorbic acid was higher in pregnant than in non-pregnant rabbits, especially in the early stages. From data obtained, it was suggested that the corpus luteum hormone may be concerned in the production of ascorbic acid, especially in those tissues where it is active. Since increases and decreases in ascorbic acid content precede corresponding changes in corpus luteum growth rate, the possibility exists that vitamin C controls corpus luteum growth.—H. O. Haterius.

A quantitative method for the bio-assay of progestin. Pincus, G., and N. T. Werthessen, Am. J. Physiol. **120**:100. 1937.

To offset the disadvantages of the several bio-assay methods extant, a quantitative scheme has been evolved, based essentially upon the observation that blastocyst growth in the rabbit is a function of corpus luteum activity. The method relies upon measurement of ovum size and the degree of glandular proliferation of the mucosa; combining the 2 sets of measurements gives a fairly precise index of hormonic activity. Animals are spayed 18 to 20 hours post-coitum, and injected subcutaneously with test material twice daily on the second, third, and fourth days, with sacrifice on the fifth day. A mean ovum size of 478.8μ or greater in a single test animal is taken to signify the presence of active progestin; a value of 0.3227 or greater for the ratio of the glandular area, G, to total mucosa area, M, and a value of 49.30 or greater for the combined index are similarly significant. In a single test animal a dosage of 0.78 mg. of progesterone can usually be detected by ovum size measurement. The action of 0.45 mg. may be detected by the G/M ratio, and by using the combined index a dosage of 0.38 mg. of hormone may be evidenced.—H. O. Haterius.

Treatment of dystrophia adiposo-genitalis with gonadotropic hormone from the urine of pregnant women. Plum, P., Acta Med. Scandinav. **93**:65. 1937.

Gonadotropic hormone derived from pregnancy urine was used in the treatment of 5 cases of dystrophia adiposo-genitalis, 2 cases of cryptorchism and 1 case of obesity. In cases of dystrophia adiposo-genitalis this treatment alone in dosages of 200 M.U. daily produced a marked growth of the genitalia, but no change in metabolism or distribution of fat. In combination with thyroid therapy a marked loss in weight was obtained. In one patient with cryptorchism but without signs of endocrine disturbances the treatment was followed by a marked growth of the genitals, but the testes did not descend after 2 months. In a second case with obesity and decreased B.M.R., descent was obtained in 2 weeks.—J. M. L.

Correlation between ocular stimulation and spermatogenesis in the English sparrow. Ringoen, A. R., and A. Kirschbaum, Proc. Soc. Exper. Biol. & Med. **36**:111. 1937.

Juvenile male sparrows exposed to artificial light for 7 hours nightly showed greatly accelerated testicular development by the 45th day of treatment. Similar birds, exposed to similar light, but with the ocular region covered by a hood, did not show this response. It is concluded that the testicular response to light in this species is largely dependent upon reception of the stimulus in the ocular region.—M. H. F.

Action of testosterone on lactation. Robson, J. M., Proc. Soc. Exper. Biol. & Med. **36**:153. 1937.

The daily injection of 0.1 mg. of testosterone-proprionate in oil inhibited lactation in mice but did not interfere with the demonstration of maternal instincts. The injection of the same material in alcohol suspension every 2 hours during the day brought about the inhibition much more quickly. The injection of androsterone in oil, or every 2 hours in the 10% alcohol suspension, did not produce similar effects.—M. H. F.

The chemical diagnosis of pregnancy by detection of estrin in urine. II. A note on the hydrolysis of estrin esters. Schmulovitz, M. J., H. B. Wylie and J. N. Cianos, J. Lab. & Clin. Med. **22**:1037. 1937.

Pregnancy urine was brought to pH 4 with hydrochloric acid, hydrolyzed, and twice extracted. The residue of urine was then made up to its original volume; brought to pH 1; hydrolyzed; and extracted. The second residue was then made up to 15% by volume with hydrochloric acid; hydrolyzed; and again extracted. Estrogenic substances were found in the subsequent extracts at the higher acidities. The additional yield was much less than that obtained in the first extraction following hydrolysis at pH 4. However, the further hydrolysis at the higher acidities produced, in addition to more free estrin, some chromogen which was confusing in the diagnosis of pregnancy in the colorimetric procedure used by the authors.—H. W. Sulkovitch.

Secretin is a true cholagogue. Tanturi, C. A., A. C. Ivy and H. Greengard. Am. J. Physiol. **120**:336. 1937.

Secretin was found to demonstrate a true cholagogue action in 4 dogs and 2 cats which had been eviscerated, with the exception of the liver and urinary bladder. The character of the biliary response is believed to preclude the possibility that the effect is due to the mechanical influence on the bile ducts.—R. R. Durant.

The sugar and cholesterol picture of blood of monkeys upon the administration of synthetic sex hormones (Kartina sachara i kholesterina krovi u obezjan pri nagruzkakh sinteticheskimi seksualjnyn mi gormonami). Tavastsherna N. I., Arkhiv Biologicheskikh Nauk **40**:137. 1935.

Injections of synthetic sex hormones (dosage 40 to 50 mg. over 3 to 5 days) were given 5 monkeys, and fasting blood cholesterol and sugar determinations made over a period of 9 days. Administration of estrone to 3 females (2 immature and 1 postmenopausal) led to gradual increment of blood cholesterol of 30 to 80 mg. over initial values. Androsterone injections into 2 male monkeys caused a slight fall in cholesterol values. On the whole the author's figures point to no significant changes. Effects on blood sugar were negligible.—B. N. Toger.

Gravimetric method for the determination of sodium pregnanediol glucuronidate (an excretion product of progesterone). Venning, Eleanor H., J. Biol. Chem. **119**:473. 1937.

Sodium pregnanediol glucuronidate was extracted from urine with normal butyl alcohol and purified by precipitation with acetone. The material was then dissolved in hot 95% ethyl alcohol, and the solution evaporated to dryness and weighed. A correction for loss was made varying from 30% for 2 to 4 mg., to 13% for 26 to 50 mg. The compound was considered an excretion product of progesterone.—J. M. L.

The determination of estrin in urine with the photoelectric colorimeter. Venning, Eleanor H., K. A. Evelyn, E. V. Harkness and J. S. L. Browne, J. Biol. Chem. **120**:225, 1937.

The estrin compounds extracted from 300 cc. of urine with butyl alcohol were hydrolyzed by autoclaving with concentrated hydrochloric acid, and re-extracted with peroxide-free

ether. The residue was dissolved in alcohol and an aliquot taken for the determination. The estrin solution was evaporated to dryness and treated with a mixture of 3.6 parts of phenol and 5.6 parts of concentrated sulfuric acid and the tube placed in a boiling-water bath for 20 min. Readings were taken through blue and green filters in the photoelectric colorimeter. The amount of estrin was determined by use of a nomogram. Between 500 and 5000 micrograms of estrin per liter of urine can be determined with a maximum error of 10 per cent.—J. M. L.

The influence of pseudopregnancy on follicular sensitivity to pregnancy urine extracts. Weinstein, G. L., and A. W. Makepeace, Am. J. Physiol. **119**:508. 1937.

Using pregnancy urine extract (antuitrin-S) standardized in M.O.D. (minimal ovulating dose) the follicular sensitivity of the ovary was studied at various stages of pseudopregnancy in the rabbit. To summarize: In 5-day pseudopregnant animals, 3 M.O.D. were required to produce 100% response; in 10-day pseudopregnancy animals, 2 M.O.D. produced 100% response; in 15-day pseudopregnant rabbits, 2 M.O.D. again evoked 100% response. Hence, at the fifth day the ovary is ½ or ⅓ as sensitive to P.U. extract as that of the postpartum rabbit, and at the 10th or the 15th day no less than ½ as sensitive as at postpartum. The appearance of large follicles on the sixth day of pseudopregnancy is correlated with an increase in follicular sensitivity, suggesting that sensitivity to P.U. extract is an index of the degree of follicular maturation existing. Some change associated with pseudopregnancy, probably the corpus luteum hormone, may apparently act partially or completely to block the postcoital discharge of the anterior pituitary and thus prevent ovulation during pseudopregnancy. The possibility also exists that the gonadotropic hormone may be secreted in quantity during pseudopregnancy, but its metabolism so changed as to inactivate or destroy it. However, this contingency seems rather unlikely.—H. O. Haterius.

Effective clinical dosages of theelin in oil: Based on study of sixteen castrate women. Werner, A. A., G. Jones, J. Roberts, G. O. Broun, C. H. Neilson and N. O. Rothermich, J.A.M.A. **109**:1027. 1937.

The authors find that theelin in oil stimulates development of the sex-related structures of the human female, producing changes in the breasts, gross appearance of the vagina, with increased mucous secretion, and growth of the endometrium and vaginal mucosa in dosages as low as 5,000 I.U. Definite changes in the vaginal smears were noted with dosages of theelin in oil as low as 10,000 I.U. Vaginal smears would appear to be a less delicate index of theelin administration than uterine mucosal specimens. Relief from symptoms of castration was obtained with dosages as low as 5,000 I.U., which is insufficient to produce the full follicular phase in the vaginal smears. This experiment proves that, when the element of time is considered, dosages of 5,000 I.U. of theelin in oil will mitigate or relieve the symptoms of castration but at the same time stimulate development of the endometrium sufficiently to cause uterine bleeding when discontinued. Theelin in oil is much more effective than theelin in aqueous solution. When administered intramuscularly in the human being, smaller dosage and less frequent intervals of injection produce more rapid and more marked effect on the endometrium and vaginal mucosa. The evidence seems conclusive that the large dosages of theelin advocated by some (from 30,000 to 50,000 R.U.) as necessary to produce the interval phase of the endometrium are grossly excessive.—Abst., A. M. A. Service.

Relation of urinary excretion of estrone to menstrual cycle of normal women. Yerby, L. D., Proc. Soc. Exper. Biol. & Med. **36**:496. 1937.

Crude estrone extracts were made from the daily 24-hour output of 2 women and the products assayed by the intravaginal method. In both women, the excretion rose from a low of 2,000 U. daily to 10 to 12,000 U. daily in two peaks—one about mid-period, and another just preceding menstruation.—M. H. F.

The influence of hormone therapy on the changes of the mucosa of the pharyngo-largyngo-tracheal tract after castration (Influenza dell'ormonoterapia sulle modificazioni della mucosa del tratto faringo-laringo-tracheale secondarie a castrazione). Zanzucchi, G. Biochem. e. terap. sper. **24**:130. 1937.

The mucosa of the upper respiratory tract of male or female rabbits undergoes atrophic changes after castration. Treatment with follicular liquid and prolan caused considerable changes in the thickness of the epithelial strata and generally a complete disappearance of the castration changes.—A. E. Meyer.

HYPOPHYSIS

Aspects of splanchnomegaly associated with the action of prolactin. Bates, R. W., O. Riddle, E. L. Lahr and J. P. Schooley, Am. J. Physiol. **119**:603. 1937.

The administration of prolactin (to completely hypophysectomized pigeons) resulted in normal body growth and excessive increase in liver weight. Three weeks after hatching, pigeons show rapid growth, crop-gland stimulation and overgrowth of the liver and intestine. This is assumed to be due to prolactin from the bird's own pituitary, since the same results are produced by the administration of prolactin to older birds. The authors conclude that these results throw doubt upon the separateness of a growth hormone in the pituitary.—F. A. Hitchcock.

Effect of infundibular puncture on glycemia and hepatic glycogen in rats (Influence de la piqûre infundibulaire, chez les rats, sur la glycémie et le glycogène hépatique). Cahane, M., Compt. rend. Soc. de biol. **125**:192. 1937.

In 9 rats, puncture of the infundibular region of the brain was performed by thermocautery through the palatine roof. This operation immediately caused a hyperglycemia which reached a maximum level in 3 hours, after which it subsided, normal sugar levels being reëstablished in 24 hours. A progressive diminution in hepatic glycogen was observed over a period of 4 months.—E. Cutuly.

Effect of pituitary substances on adrenalectomized rats. Cavanaugh, C. J., and R. Gaunt, Proc. Soc. Exper. Biol. & Med. **37**:226. 1937.

Although earlier experiments indicated that large doses of pituitary implants did not increase survival time of adrenalectomized young rats, it was found that the implantation of single whole male rat pituitaries twice weekly following adrenalectomy in 30-day-old female rats definitely prolonged survival. However, despite continued treatment, the rats succumbed with deficiency symptoms after about 18 days. Negative results were obtained with beef pituitary implants in similar dosage, and with various hormonal preparations. Progesterone at the level of 0.15 I.U. per day was ineffective. Injections of 25 to 50 U. of estrin daily shortened the life of the adrenalectomized animals.—M. H. F.

A change in the basophil cells of the pituitary gland in common to conditions which exhibit the syndrome attributed to basophil adenoma. Crooke, A. C., J. Path. & Bact. **41**:339. 1935.

A hyaline change in the basophilic cells of the pituitary gland is described which appears to be an expression not of cell degeneration in the ordinary sense but probably of altered physiologic activity. The hyalin was observed in slight amount in a few basophilic cells of the anterior lobe in only 9 of about 350 pituitary glands of persons with various conditions in which the syndrome attributed to basophil adenoma was absent. Its presence in examples of the different conditions in which it was present was inconstant and exceptional. It was observed in large amount in many basophilic cells in the acini of the anterior lobe in all 12 available examples of the syndrome attributed to basophilic adenoma, a neoplasm of the thymus or a neoplasm or hyperplasia of the adrenal cortex. Analysis shows that this conspicuous amount of hyaline change was the only abnormality common to all 12 examples of the syndrome. The conclusion is that it is the abnormality of fundamental significance.—Author's Summary.

Resynthesis of muscle glycogen in hypophysectomized toads (Recomposición del glucógeno muscular en el sapo hipofisoprivo). Dambrosi, R. G., Rev. Soc. argent. de biol. **13**:27. 1937.

The resting muscle glycogen of toads 14 days after hypophysectomy was found to be approximately one-half (453 mg.%) that in normal controls (806 mg.%). After 15 m. strong tetanus of the hind legs (paradisation of the sciatic nerves), the muscles of one side were extracted, those of the other were examined 1 to 5 hours later. The resynthesis of glycogen did not occur as in the normal controls, even after 5 hr. Subcutaneous implantation of the principal lobe of the hypophysis was performed in the operated animals during 6 days beginning 14 days after operation. Twelve hr. after the last implantation the muscles were fatigued and the glycogen resynthesis was found to be almost as complete as in the normal controls. Treatment with insulin did not improve the condition, and an injection given after fatigue diminished the muscle glycogen even more.—J. T. L.

Effect of adrenotropic extract upon the accessory reproductive organs of castrated rats. Davidson, C. S., Proc. Soc. Exper. Biol. & Med. **36**:703. 1937.

The injection of adrenotropic pituitary extracts into castrate rats, with and without previous hypophysectomy, led to a marked increase in the weights of prostate and seminal vesicles. Such enlargement did not occur in the absence of the adrenals.—M. H. F.

Influence of infections, toxins and poisons on hypophyseal gonadotropic activity (Influencia de las infecciones, toxinas y tóxicos sobre el poder gonadotrópico hipofisario). Del Castillo, E. B., Rev. Soc. argent. de biol. **13**:163. 1937.

Male rats (16) received 50 mg. per kg. Fl daily during 30 days; the hypophysis implanted in infantile rats produced an increase in weight of ovaries similar to that produced by the hypophysis of the controls. Thalium acetate injected twice weekly during 40 days in doses of 4 mg. (15 males) did not modify the effect of the pituitary on ovarian weight of prepubertal rats, as compared with that of controls. Male (12) and female rats were inoculated with a strain of Trypanosoma equiperdum that killed in 10-12 days; at the 10th or 12th day when they were killed and the gonadotropic activity of the hypophysis was tested, no difference from that of the controls was found. Intoxication with diphtheria toxin did not alter the gonadotropic activity of the hypophysis of dogs.—J. T. L.

Prolongation of action of pituitary antidiuretic substance, and of histamine by metallic salts. Dodds, E. C., R. L. Noble, H. Rinderknecht and P. C. Williams, Lancet **233**:309. 1937.

These observers report that zinc salts, for some unknown reason, prolong the antidiuretic activity of the posterior pituitary products, as well as prolonging the action of histamine in the stimulation of gastric secretion.—L. B. S.

Effects of hypophyseal extract and of adrenaline on the pigmented epithelial fringes of the retina in tadpoles (Effets de l'extrait hypophysaire et de l'adrénaline sur les franges de l'épithélium pigmentaire de la rétine de la grenouille). Dubois-Poulsen, A., Compt. rend. Soc. de biol. **125**:248. 1937.

One group of tadpoles was exposed to light and a second group was kept in darkness. Injections of post-hypophyseal substance in the latter group caused descent of the pigmented fringes of the retina; adrenaline caused a similar but more vigorous response. In the group exposed to light, posterior hypophyseal substance also caused descent of the retinal fringes, while adrenaline brought about their retraction.—E. Cutuly.

Treatment of Cushing's syndrome with large doses of oestrin. Gill, A. M., Lancet **233**: 72. 1937.

Three cases of Cushing's syndrome were given large doses of estrin, namely, 9,300,000 U over a period of 50 days, 8,600,000 for 43 days, and 9,000,000 U for 45 days, respectively. No effect was obtained on the obesity, hypertrichosis, abdominal striae, or hypertension. Subjective improvement in the severe headaches were noted.—L. B. S.

Oestrogenic treatment of hypophysectomized male mice. Gomez, E. T., C. W. Turner, W. U. Gardner and R. T. Hill, Proc. Soc. Exper. Biol. & Med. **36**:287. 1937.

The daily injection of 150 I.U. of estrin, in various forms for 25 to 214 days, did not stimulate the mammary glands in the animals, if the hypophysectomy had been complete, as demonstrated by serial section. However, if a small fragment of pituitary remained, insufficient to prevent atrophic changes in the sex gland and adrenals, such fragment enabled the animal to respond almost normally to the estrin injections by mammary gland proliferation. Resorption of the pubic symphysis occurred, in the complete absence of the pituitary in the treated animals—M. H. F.

Organic changes in guinea pigs and rats following thyreotropic hormone (Experimentelle Untersuchungen an Meerschweinchen und Ratten zur Frage der Organveränderungen durch thyreotropes Hormone). Heinemann, K., Endokrinologie **19**:1. 1937.

Intra-peritoneal injection of thyreotropic hormone into guinea pigs and rats calls forth the signs of Basedow's or Graves' disease as seen in man. The accompanying changes in the endocrine organs are inconspicuous. Lacking also are the marked degenerative changes in the heart and the liver as described for man.—W. J. A.

Androgens and corpus luteum formation (Mannliche Wirkstoffe und Corpus Luteumbildung). Hohlweg, W., Klin. Wchnschr. **16**:586. 1937.

The daily injection of androsterone in 3 mg. doses for 8 days, or in 15 mg. doses for 8 days did not cause the appearance of estrus in 50 gm. female rats, nor the formation of corpora lutea. Yet similar injections of dehydroandrosterone led to both estrus and corpus-luteum formation, although daily injections of 5 mg. of testosterone for 8 days in similar animals led to the formation of corpora lutea without the appearance of estrus. From such experiments, the author concludes that the ability of a compound to produce corpora lutea in the immature rat is not related to its ability to prevent castration changes in the pituitary, or its estrogenic properties.—M. H. F.

Hypophyseal thyrotropic mechanism essential for occurrence of diabetes insipidus in its maximal form. Keller, D., Proc. Soc. Exper. Biol. & Med. **36**:787. 1937.

The injection of anterior lobe extract given a dog, in which hypophysectomy had abolished a diabetes insipidus previously produced by an hypothalmic lesion, again produced marked polydypsia 7½ months later. However, with continued injections the effect wore off, and even with a doubled dose of the extract the water intake remained normal until the oral administration of thyroid (0.1 to 2.4 gm.) again led to the polydypsia. It is concluded that the rôle of the anterior pituitary in the development of diabetes insipidus is through the thyroid by way of the thyrotropic principle.—M. H. F.

Hypophyseal transplantation—experimental and clinical (Hypophysentransplantationem. Tieresperimentelle Untersuchungen und klinische Ergebnisse). Kylin, E., Acta med. Scandinav. **91**:428. 1937.

Small pieces of calves' hypophyses were transplanted into peritoneal pockets of rabbits. After 1½ to 3 months the rabbits were killed and the tissues examined. In all transplants the central portions were necrotic, but the peripheral portions showed all 3 types of normal cells. The pieces which had no capsule adherent did better than those in which it remained, and smaller pieces took better than large ones. In 28 cases of Simmonds' disease treated by transplantations of calves' hypophyseal glands, 5 were too recent to judge the results, 2 died within 3 months, 15 were healthy and able to work, 4 were significantly improved, and 2 were unchanged. Improvement in most cases had lasted for 2 years.—J. M. L.

Maternal behavior in hypophysectomized male and female mice. Leblond, C. P., and W. O. Nelson, Am. J. Physiol. **120**:167. 1937.

Observations were made upon hypophysectomized lactating females, upon hypophysectomized young males and females, a 'maternal index' being devised to serve as a criterion of degree of parental behavior. Hypophysectomy in the lactating female, while it stopped lactation, had relatively small or a temporary effect in decreasing the value of the maternal index. Since the factor of conditioning might plausibly account for its continuance in the mother, immature animals, 24 to 40 days old, were employed. Hypophysectomy was followed by placing in contact with young at suitable intervals. Such animals when placed with new-born mice for several days developed maternal behavior; similar observations were made with rats. Two normal 50-day old mice failed to develop indications of maternal behavior when placed with young, either before or after hypophysectomy. Ten U of lactogenic hormone (prolactin, Squibb) daily for 10 days failed to elicit a positive response. The writers believe a nervous mechanism to be prepotent in developing the maternal behavior of rats and mice, superimposed upon which hormonic factors may be involved at the time of parturition.—H. O. Haterius.

Prolactin-like reaction produced by hypophyses of various vertebrates. Leblond, C. P., and G. K. Noble, Proc. Soc. Exper. Biol. & Med. **36**:517. 1937.

By the local implantation technic, crop-gland responses were obtained with the pituitaries of birds, reptiles and amphibians and fish. However, since some response was also obtained by the implantation of liver and brain, it could not be regarded as specific.—M. H. F.

Augmentation of the gonadotropic hormone from the pregnant mare. Lein, A., Proc. Soc. Exper. Biol. & Med. **36**:609. 1937.

The injection of the luteinizing fraction of sheep pituitaries, together with pregnant mare gonadotropin, resulted in typical augmentation as shown by the ovarian weights of the injected animals (21-day mice). This augmentation was not observed if the pituitary preparation had first been boiled, nor was it obtained with muscle extracts containing equal amounts of protein.

It is concluded that the augmentation observed is dependent on hormonal action, and is not a non-specific effect of the inert materials.—M. H. F.

Some observations on intermedin. Lewis, D., F. C. Lee and E. B. Astwood, Bull. Johns Hopkins Hosp. **61**:198. 1937.

By modifying the technic of Zondek and Krohn, who used the erythophore expanding property of elritze to determine the pressure of intermedin, it was possible to obtain much higher values for intermedin in various parts of the hypophysis of cattle. Some preparations yielded 5 U per gamma. A very high concentration of intermedin was found in the cleft exudate. Probably because of diffusion, a lesser concentration of intermedin was found the further the samples of tissue were taken from the pituitary gland. Due to the absence of typical basophilic cells in the pars intermedia of cattle, it is felt that intermedin is probably not formed by these cells.—Authors' Abst.

Rôle of pituitary in effect of testosterone on the mammary gland. McEuen, C. S., H. Selye and J. B. Collip, Proc. Soc. Exper. Biol. & Med. **36**:213. 1937.

The daily injection of 200 gamma of testosterone in oil given hypophysectomized male rats for 19 days had no effect on the mammary gland, whether the treatment was begun immediately postoperatively, or after an intervening period of 21 days. Neither did the injection of PU into such animals stimulate the mammary gland, although the injection of anterior lobe extracts caused testicular growth and mammary gland stimulation. In the course of the experiments it was also noted that the testosterone therapy did not prevent testicular atrophy following the operation. It is concluded that testosterone does not stimulate mammary gland development in hypophysectomized animals.—M. H. F.

Heme containing fractions of blood as related to the augmentation of pituitary gonadotropic extracts. McShan, W. H., and R. K. Meyer, Am. J. Physiol. **119**:574. 1937.

To determine the substances in the formed elements of blood responsible for the reported augmentation of pituitary gonadotropic extracts a study was made of the action of cow's blood, erythrocytes, hemoglobin, globin, porphyrin, heme and hemin in conjunction with two types of APE, an acetone soluble fraction of a pyridine extract of sheep glands (Fevold et al) and a water soluble fraction of whole gland. A given quantity of the separated blood substances was admixed in vitro with a definite amount of the gonadotropic extract. The material was given subcutaneously to 21-day old female rats. Whole blood, hemolyzed erythrocytes, hemoglobin, heme and hemin were found to augment the activity of gonad-stimulating extracts. By elimination, the writers believe that heme is the active augmenting constituent, and that the activity of the other substances results from their breakdown into globin and heme, the latter combining with the pituitary principle. Greatest increase in mean ovarian weight occurred following administration of 8 mg. heme and extract. Globin prepared from hemoglobin gave no significant augmentation. Inorganic iron failed to augment. Heme injections alone failed to augment, and separate injection of hemin and of gonadotropic hormone was ineffective. This suggested the possibility of a combination of heme with the gonadotropic complex in vitro, which is not found to occur in the body when they are administered separately. The explanation for this in vitro augmentation is not known, but delay in absorption, generally invoked to account for augmentation phenomena, seems improbable as a prime factor, since there was no evidence of the formation of an insoluble gonadotropic complex when heme and extract were mixed.—H. O. Haterius.

Influence of extracts of anterior lobe of pituitary on glucose oxidation and glycogen storage. H. S. Meyer, L. J. Wade and C. F. Cori, Proc. Soc. Exper. Biol. & Med. **36**:346. 1937.

In rats fasted 24 hours, the administration of anterior lobe extract intraperitoneally 1½ to 3 hours before the administration of glucose by stomach tube decreased the amount of sugar metabolized, and increased the deposition of liver and muscle glycogen as shown by Haldane gravimetric metabolism determination, blood and urine sugar examinations, and glycogen analysis in liver and muscle. Heated anterior lobe extract did not have this effect.—M. H. F.

The effect of hypophysectomy on arterial blood pressure of dogs with experimental hypertension. Page, I. H., and J. E. Sweet, Am. J. Physiol. **120**:238. 1937.

Chronic hypertension induced in dogs by constriction of the renal arteries was compensated by hypophysectomy. The fall in blood pressure does not occur suddenly, and constriction of the arteries following hypophysectomy causes only a transitory rise. It is postulated that hypo-

physectomy alters the reactivity of the vascular system by diminishing the activity of the adrenal cortex and thyroid, so that it no longer responds as well to chemical stimuli resulting from constriction of the renal artery.—R. R. Durant.

The presence of prolan in the urine of a patient whose pituitary was completely destroyed by a metastatic tumor (Constatation de l'existence de prolan dans l'urine d'un malade dont l'hypophyse était complètement détruite par une tumeur métastatique). Mussio-Fournier, J. C., W. Buno, J. Morato-Manaro and A. Albrieux. Presse méd. **45**:531. 1937.

The pituitary tissue was completely destroyed and replaced by malignant growth, a metastasis from a tumor of the testicle. The urine contained 200 R.U. of prolan. The theory advanced is that the hormone is formed in the tumor tissue.—A. E. Meyer.

The value of the Aschheim-Zondek reaction in the diagnosis of brain tumors (La valeur de la reaction d'Aschheim-Zondek dans le diagnostic des tumeurs cérébrales). Monnier, M., Presse méd. **45**:412. 1937.

Tumors in the diencephalon (third ventricle) and in the frontal lobe cause stimulation of the hypophysis. The Aschheim-Zondek reaction was found positive in 50% of cases of cerebral tumor. This is true only so long as the connection between brain and gland is not destroyed. The cerebro-spinal fluid does not contain more of the pituitary hormone than is found in the urine.—A. E. Meyer.

The influence of anterior pituitary grafts on the development of the chicken embryo (Esperienze di innesota di ipofisi anteriore sullo sviluppo dell'embrione di pollo). Pighini, G., Biochim. e. terap. sper. **24**:187. 1937.

Anterior pituitary from chicken introduced into the fertilized chicken egg after 5 days of incubation, accelerated the development of the embryo.—A. E. Meyer.

Successful treatment of schizophrenia in a general hospital. With a résumé of the technic employed. Rea, C., and C. M. Kershner, Pennsylvania M. J. **41**:14. 1937.

This report shows that treatment for schizophrenia can be successfully carried out in a general hospital without the trained personnel or equipment of a psychopathic ward. It is imperative that the physician in charge have full and complete knowledge of the method and the reactions to be expected. Preferably he should have the experience of personal observation of several cases so treated or, lacking the opportunity for this, he must have a background of wide experience with the reactions of insulin fortified by extensive reading of the foreign literature on the Säkel method. Nurses in charge of the patient should be the most competent and trustworthy obtainable and should have psychiatric experience. The treatment is complicated, difficult, dangerous, and necessarily expensive; nevertheless the method is justified by the success obtained in a hitherto almost hopeless disease. Incidentally, as has been suggested before, the authors see no reason why the method should not be tried and possibly proved successful with other types of purely mental disease.—I. B.

Observations pertinent to gonadotropic therapy in gynecology. Ross, R. A., Am. J. Obst. & Gynec. **34**:780. 1937.

Summarizing 7 years' experience, using both APL and pituitary gonadotropic therapy, the results with 85 patients are tabulated. There were no appreciable effects in cases with amenorrhea, in those before the menarche or after the menopause. Eight menorrhagics had clinical benefit, but without demonstrable formation of a functional corpus luteum. The effects of APL appear more like those seen in pregnancy than in the normal cycle. The author feels that the need is for higher potency preparations of pituitary gonadotropic extracts. The treatment lasted usually for 1 to 6 days only, with a few of the patients treated longer: only 2 for over 12 days.—E. L. S.

Hypophyseal infantilism: treatment with an anterior hypophyseal extract; final report. Shelton, E. K., L. A. Cavanaugh and H. M. Evans, Am. J. Dis. Child. **52**:100. 1936.

This is the second, and perhaps the final, report of a study of a group of 6 dwarfish children who have been under observation for nearly 5 years. Five of the patients received treatment of intramuscular injection of alkaline extracts of the hypophysis for varying periods. One had never received such treatment. One died as a result of recurrent cyst of the supracellar pouch. The authors conclude that APE will promote growth in human subjects when administered intramuscularly; the stimulation of growth appears to be in direct ratio to the amount

of extract used. From a clinical viewpoint, however, the growth-promoting property is disappointing; the preparations are not sufficiently active to rehabilitate the classical dwarf, but they may be useful during the growing period of dwarfish persons who are losing not over 50% of the normal growth increment. Prolonged administration produces little evidence of a subsequent refractory state, and does not interfere with either the onset of puberty or the spurt of growth which may occur in this period.—M. B. G.

Carbohydrate metabolism hormone of anterior hypophysis (Zur Frage des Kohlenhydratstoffwechselhormons des Hypophysenvorderlappens). Singer, K., and M. Taubenhaus, Wien. Arch. f. inn. Med. **31**:59. 1937.

The carbohydrate metabolism hormone of Anselmino and Hoffmann was tested by using rabbits instead of rats. With rabbits a marked uniform lowering of liver glycogen was not obtained. The authors conclude that the existence of a definite carbohydrate-metabolism hormone of the anterior hypophysis has not been proved.—W. J. A.

Influencing duration of pregnancy by anterior hypophyseal hormone (Beeinflussung der Schwangerschaftsdauer durch Hypophysenvorderlappenhormone). Spreng, A., Endokrinologie **19**:161. 1937.

Seven pregnant rats were injected with 40 or 80 R.U. of antuitrin-S on the 15th day *post-coitum,* and the effect on the duration of pregnancy noted. Contrary to the findings of Snyder for the rabbit, it was found that antuitrin-S does not regularly prolong pregnancy in the rat, since in 2 animals the time of gestation was not modified; in 3 rats delivery followed 3 to 4 days after the injection; and in 2 pregnancy was prolonged for 3 to 4 days. The large number of stillbirths from the experimental animals as contrasted with the controls is cited as evidence for a toxic action of the preparation.—W. J. A.

The treatment of hypogenitalism in the male. Thompson, W. O., N. J. Heckel, A. D. Bevan and P. K. Thompson, Illinois M. J. **72**:279. 1937.

The authors observed marked stimulation of genital growth from the use of APL principle in the treatment of boys with undescended testes and hypogenitalism. However, premature puberty may result if treatment is continued too long. Since this material is a powerful therapeutic agent, its administration should be carefully supervised.—I. B.

Ovarian weight responses to menopause urine injections in normal hypophysectomized and hypophysectomized thyroxin-treated immature rats. Tyndale, H. H., and L. Levin, Am. J. Physiol. **120**:486. 1937.

Doses of menopause urine extract which gave marked follicular enlargement, and little or no grossly observable luteinization in the immature rat ovary, resulted in the production of much heavier ovaries in hypophysectomized animals than in unoperated controls. With higher dosage giving luteinization in the normal but not in the hypophysectomized rats, it was found that the ovarian weight response was reversed, the normal ovaries being the heavier. The greater ovarian weight, obtained in the hypophysectomized rat during the follicular phase of ovarian development, indicates that an inhibitory factor is operative in the normal animal. That this factor could be the greater activity of the thyroid gland is evident from experiments in which the ovarian follicle stimulation induced by extracts of menopause urine in immature hypophysectomized rats has been markedly decreased with the simultaneous injection of thyroxin. These latter experiments indicate that the inhibiting action of thyroid hormone upon the mechanism of gonad-stimulation is a direct one and not mediated through the pituitary gland, since the experiments were performed on hypophysectomized animals.—Authors' Abst.

Stimulation of anterior hypophysis metabolism by theelin or dihydrotheelin. Victor, J., and Dorothy H. Andersen, Am. J. Physiol. **120**:154. 1937.

The in vitro and in vivo effects of theelin and of dihydrotheelin upon respiration of the anterior pituitary were observed in spayed rats. Of 3 tissues studied (pituitary, liver, kidney) only hypophyseal substance was affected, i.e., in an increased rate of oxygen consumption of the tissue within one hour. Similar in vitro experiments with l-thyroxin failed to demonstrate stimulation of any of the tissues studied. The intraperitoneal injection of an aqueous solution of 4 gammas of dihydrotheelin increased the rate of oxygen consumption of the pituitary within 6 hours, resumption of the normal level occurring within 12 hours. Since neither liver nor kidney tissue reacted in the same manner, the reaction was regarded as a specific one. The authors feel it probable, after comparing changes of the cytology, metabolism, and gonad-

stimulating potencies following administration of estrogenic substances, that the heightened metabolism, degranulation of basophils, and increased gonadotropic activity are related. Since dihydrotheelin or theelin stimulate metabolism of the anterior pituitary of spayed rats, and since estrin tends to degranulate the basophils and briefly increases gonadotropic activity of the gland (Hisaw), it would appear that spaying removes the stimulus (dihydrotheelin) for degranulation, which results in an increase in granules. Moreover the changes in rate of elaboration of dihydrotheelin coincident to changes in the follicles during the estrous cycle and at parturition appear to result in liberation of the luteinizer, with increase in metabolism and accompanying degranulation.—H. O. Haterius.

Pituitary gland influences on water balance in the rat. White, H. L., Am. J. Physiol. **119**:5. 1937.

This work is a confirmation and extension of the work of Pencharz and collaborators. The effect of beef anterior lobe extract, thyroid and thyroid plus anterior lobe extract on normal, postlobectomized and hypophysectomized rats was studied. Anterior lobe extract alone, and thyroid alone, produced no increase in urine volume; anterior lobe and thyroid extract produced no change except an increased urine volume in one case out of 7 hypophysectomized rats. The lack of increased urine volume cannot be ascribed to lack of increase in B.M.R., as oxygen consumption was followed in the various series. The lack of response of the rat is similar to that found in the monkey.—Lena Lewis.

Gonadotropic hormones of the hypophysis of the turkey. Witschi, E., A. J. Stanley and G. M. Riley, Proc. Soc. Exper. Biol. & Med. **36**:647. 1937.

Acetone-dried pituitaries from male and female turkeys were taken up in distilled water and injected into normal and hypophysectomized rats, and into sexually quiescent male and female sparrows. In both kinds of rats, the equivalent of 1 to 4 pituitaries produced results similar to those obtained from mammalian pituitaries, i.e., follicular growth and corpora-lutea formation. Similarly, the extracts produced marked stimulation in the testes and ovaries of injected sparrows, thus differing from the results obtained with human pregnancy preparations. On the basis of potency per unit of dry weight, the turkey pituitary appears to be more potent than bull, but less potent than sheep or rat pituitary.—M. H. F.

Hyperinsulinism. Final report of case including necropsy observations. Ziskind, E., W. Bayley and E. F. Mauer, Arch. Int. Med. **60**:753. 1937.

The case described was that of a young man of 19 who was admitted in a state of coma to the Los Angeles County General Hospital on September 8, 1930. For 18 months he had had spells of weakness, headache, trembling and mental confusion which occurred at about noon. The sugar content of the blood was found to be 40 mg. per 100 cc. He remained in coma for 10 days. He was observed for 4 years (most of the time in a hospital). Necropsy revealed an adenoma of the pancreatic islets which had not been discovered at 2 previous pancreatic resections. Dinitrophenol had a retarding effect on the increasing obesity. This result and the physiologic indications for the drug in these cases would indicate its further trial for symptomatic relief, particularly in hypoglycemia not amenable to surgical intervention, were it not for the severe toxic effects sometimes noted when this drug is used. A rise in the content of inorganic phosphates in the blood was discovered during the hypoglycemia present after the ingestion of dextrose. Impairment of psychologic functions, e.g., an effect on the memory span, was among the earliest signs of insulin hypoglycemia. In this patient, the latent period between the drop in the sugar content of the blood and the ultimate fall in the sugar content of the spinal fluid was so long that the level in the spinal fluid during part of this interval was at a higher level than that in the blood. The intradermal saline test did not reveal a state of dehydration in the skin during the hypoglycemic phase.—I.B.

PANCREAS

Vagal influence on insulin secretion through vitamins B and C. (Beeinflussung der Langerhansschen Inslen durch den Vagus mittels der Vitamins B und C.) Aszodi, Z., and H. Mosonyi, Klin. Wchnschr. **28**:1214. 1937.

The intravenous injection of 300 U. of Vitamin C led to a fall in blood sugar more marked in dogs after partial pancreatectomy (mildly diabetic) than in normals. Moreover, the simultaneous administration of such dose of vitamin C along with sugar by mouth led to markedly increased sugar tolerance as evidenced by the blood sugar curves. This was true in the operated

animals as well as in the normals. Moreover, the blood of the animals injected with large doses of vitamin C showed increased insulin content, compared to control values obtained just before the injection. This was determined by blood sugar tests on mice injected intraperitoneally with the blood in question. However, not all of the effects on blood sugar could be ascribed to increased insulin secretion, in so far as completely depancreatized dogs showed some hypoglycemic response, although not so marked. Likewise, in these animals the latent period of the reaction was longer, with no sharp drop in blood sugar during the first hour as in the normal animal. All these effects were still present in the dogs immediately after bilateral vagisection, but not 24 hours later. Similar experiments performed with Vitamin B will be reported later.—M. H. F.

The effects of irradiation of the thymus on the structure of the pancreas (Gli effetti dell'irradiazione del timo sulla struttura del pancreas). Bentivoglio, G. C., and C. Fumi, Sperimentale, Arch. di. biol. **91**:219. 1937.

The involution of the thymus produced in dogs of 40-60 days by x-ray treatment is associated with an increase of the islands of Langerhans.—A. E. Meyer.

On the survival of the completely depancreatized dog. Chaikoff, I. L., and A. Kalan, J. Nutrition. **14**:459. 1937.

When maintained with insulin and a high-calorie, high-protein, high-vitamin diet, completely depancreatized dogs may survive for as long as 4 to 5 years. The pathological changes observed in these animals during survival are recorded. This length of survival makes it unnecessary to assume that raw pancreas or extracts thereof or choline supplements (i.e., in addition to that contained in the dietary constituents) are essential for the survival of the completely depancreatized dog receiving insulin. The previous failures to obtain lengthy survival of completely depancreatized dogs maintained with insulin and a diet containing no pancreas are examined and shown to be of questionable significance in view of the inadequate diets employed.—Authors' Abst., Wistar Bib. Service.

Central nervous system and sugar metabolism. Clinical, pathologic and theoretical considerations, with special reference to diabetes mellitus. Vonderahe, A. R., Arch. Int. Med. **60**:694. 1937.

The proper understanding of diabetes mellitus as a clinical entity requires the inclusion of the central nervous system. Clinical and pathologic data indicate that the nervous system becomes an integral factor in the cycle of events which constitute the phenomena of this disease. A hypothesis is proposed which assumes that the nucleus paraventricularis in the hypothalamus is stimulated by the presence of sugar to activate the cells of the islands of Langerhans to the production of insulin, and an analogy is suggested between this mechanism and the stimulation of the cells of the respiratory center by carbon dioxide. This hypothesis has been applied to patients with diabetes mellitus without gross cerebral lesions, and to patients with various lesions of the brain, confirmed at autopsy, and presenting in common hyperglycemia or glycosuria.—I. B.

The local effects of androgens (Die Örtliche Wirkung von Sexualhormonen). Voss, H. E., Klin. Wchnschr. **16**:769. 1937.

Various androgenic materials were assayed by the local application of the substance in oil in 0.1 cc. quantities to the cock's comb. The unit was defined as that daily dose which, after 5 days' application, would produce a planimetric increase of 30% in the comb area by the seventh day. Compared to the potencies obtained with the injection technic, the various androgens were from 5 to 90 times more potent when applied locally. Moreover, some compounds, such as androstendion, and isodihydro-androsteron, which are not very active when injected, are highly potent upon local application. It is indicated that the order of potency obtained by intramuscular injection is not at all the order obtained upon local application. —M. H. F.

A study of the blood sugar raising substance in the urine of diabetic and non-diabetic patients. Werch, S. C., and S. S. Altshuler, Am. J. Physiol. **118**:659. 1937.

The study was initiated to investigate the urines of non-diabetic patients, controlled diabetics using insulin, those not requiring insulin, and uncontrolled diabetics, for a blood-sugar raising substance, and to study its properties if found. Extracts were made of the urines of these 4 types of patients according to the method of Houssay and Biosotti. Injections were made into dogs and rabbits; 2 cc. portions, which represented a constant percentage of the

whole sample were uniformly used. Blood sugar readings of the 1 hr. specimens were compared with samples taken just previous to injection. A blood-sugar raising substance was found in the urines of severe diabetics, and to a lesser degree in those of mild cases. Little or none of the substance was found in urine from non-diabetics. The factor appears not to be a protein, is absorbed by kaolin and charcoal, is soluble in H_2O and 60% C_2H_5OH, insoluble in fat solvents, and is destroyed by boiling and does not pass through a Berkefeld filter.—K. A. Brownell.

Influence of fat on concentration of sugar in blood and in urine in diabetes mellitus. Wishnofsky, M., A. P. Kane and W. C. Spitz, Arch. Int. Med. **60:**837. 1937.

Experiments are presented from which it is concluded that fat does not require insulin for its metabolism, and that it does not inhibit or retard the action of insulin. Incidentally, it is shown that the ingestion of large amounts of fat does not influence the fasting level for blood sugar of diabetic patients or normal persons.—I. B.

The course of blood sugar after intravenous insulin in normal dogs and cats. Zucker, T. F., and B. N. Berg, Am. J. Physiol. **119:**531. 1937.

The purpose of the work was to compare the typical blood sugar curve of the dog, after intravenous injection of insulin, with that of the cat. Thirty-seven dogs and 36 cats were used in the experiment, and doses of 0.1, 0.5, 1.0 and 2.0 U per kg. of Lily's clinical insulin were employed. Blood sugars were determined at 15 min. intervals, by the Schaffer-Hartman method. The dogs showed a rapid fall with a 15-30 mg. bump in the curve at 20-40 min. Cats did not show this bump but did exhibit an initial hyperglycemia with ordinary clinical insulin which was eliminated if crystalline insulin was used. In the dog, the size of the dose reflected itself nearly exclusively in duration of action. In the cat, both duration and intensity of action varied with dose. The dog showed marked oscillations of blood sugar which were absent in the cat. Insulin sufficiently purified to show no initial hyperglycemia in dogs still contained enough impurity to give an initial blood sugar rise in cats. About 1 gamma of impurity per kg. produced measurable effects.—K. A. Brownell.

THYROID

The effect of thyreotropic hormone combined with small amounts of iodine upon the function of the thyroid gland. Anderson, Evelyn M. and H. M. Evans, Am. J. Physiol. **120:**597. 1937.

Experiments carried out on both rats and guinea pigs showed that the administration of potassium iodide to both normal and hypophysectomized animals, following the injection of thyreotropic hormone, inhibited any increase in oxygen consumption, although the thyroid glands showed hyperplasia. It is concluded that the action of iodine in these experiments is similar to that concerned in the therapeutic use of iodine in Graves's disease.—F. A. Hitchcock.

The effect of thyrotropic pituitary hormone on the oxygen consumption of thyroid tissue in vitro. Anderson, R. K., and H. L. Alt, Am. J. Physiol. **119:**67. 1937.

The addition of thyrotropic hormone to slices of thyroid tissue in vitro caused an immediate increase in oxygen consumption (17 to 53%) with a progressive increase for 2 hours or more.—F. A. Hitchcock.

Thyroid and growth of the penis in drakes (Thyroïde et croissance du pénis chez le canard domestique). Benoit, J., Compt. rend. Soc. de biol. **125:**461. 1937.

An inferior development of the penis was found in thyroidectomized drakes as compared with normal drakes, comparison being made among animals with equivalent testicular size. Small amounts of regenerated thyroid tissue permitted the penis to grow normally. The significance of the thyroid in genital development is discussed.—E. Cutuly.

The heart in hyperthyroidism. Ernestene, A. C., Internat. Clin. **4:** (series 47) 78. 1937.

This is a good review of the cardiovascular problems encountered in hyperthyroidism, with special reference to roentgenologic and electrocardiographic observations, the cardiac arrhythmias, and the association of congestive heart failure, angina pectoris and cardiac asthma. The treatment recommended is subtotal thyroidectomy during an iodine remission.—I.B.

The cytoplasmic constituents of cells from the normal thyroid and those from cases of exophthalmic goiter (Sur les constituants cytoplasmiques des cellules de la thyroide normale et des goitres exophthalmiques). Feyel, P., and J. Varangot, Compt. rend. Soc. de biol. **124:**1050. 1937.

Five normal thyroids and 25 from patients with Basedow's disease were examined. The types of cell found in the normal gland are described and pictured. The difference between the normal and the goitrous thyroid was in the relative number of the different types of cells and not in any demonstrable change in their individual character.—J. C. D.

Thyroid-pituitary relationship in diabetes inspidus. Findley, T., Jr., Ann. Int. Med. **11:** 701. 1937.

The effect of thyroidectomy on water, sodium chloride and nitrogen metabolism in a case of diabetes insipidus is described. The operation was followed by definite increase in reactivity to pitressin and diminished diuretic response to sodium chloride. While on high salt diet dessicated thyroid produced a greater urine volume response than did nitrophenol, which was interpreted as due to an action different from the elevation of metabolic rate. Since salyrgan caused the elimination of urine rich in chloride, it was concluded that, if diabetes insipidus is due to inability of the kidneys to concentrate urine, the condition is reversible.—R. R. Durant.

Transplantation and retransplantation of the thyroid in the anterior chamber of the eye (Transplantation et retransplantation de la thyroïde dans la chambre antérieure de l'oeil). Goldner, J. and I. Frasin, Compt. rend. Soc. de biol. **125:**210. 1937.

Transplants of thyroid gland re-transplanted into the anterior chamber of the eyes of rabbits presented histological modifications resembling in some respects those occurring in *in vitro* explants. In addition, it was observed that thyroid tissue became activated in the presence of histiocytes, and that bridges of thyroid cells bounded on one side by a capillary and on the other by connective tissue (inverted follicles) were not viable.—E. Cutuly.

Effect on the metabolism of guinea pigs of thyrotropic hormone and of thyroxin followed by thyrotropic hormone (Action de l'hormone "thyréotrope" et de l'administration successive de thyroxine et d'hormone "thyréotrope" sur le métabolisme du cobaye). Mahaux, J., Compt. rend. Soc. de biol. **125:**379. 1937.

Thyrotropic hormone administered to guinea pigs caused an initial rise in metabolism and exophthalmos. This was followed by a relatively early drop in metabolism, but also by a slow disappearance of exophthalmos. Guinea pigs previously treated with thyroxine and then with thyrotropic hormone failed to undergo an elevated metabolism, but did show exophthalmos. The author discusses the idea that thyrotropic hormone affects metabolism indirectly through the hypothalamic nerve centers, and that thyroxine physiologically blocks these centers.—E. Cutuly.

Relation of thyrotoxicosis to emotions. McMahon, A. South. M. J. **30:**996. 1937.

This is a discussion of symptoms seen in 50 cases without specific data.—J. C. D.

The removal of sodium cholate from the blood and its secretion into the bile as affected by thyroxine. Schmidt, L. H., Am. J. Physiol. **120:**75. 1937.

Using rabbits as the experimental animal, it was shown that the removal of injected sodium cholate was delayed by the previous injection of thyroxine. The delay was longer in animals receiving larger doses of thyroxine. The thyroxine was without effect on the excretion of sodium cholate in the urine, but thyroxine treated animals were unable to secrete as large a volume or as concentrated bile as normal animals. It is suggested that thyroxine treatment causes a mild liver injury.—F. A. Hitchcock.

The relation of amniotin to the basal metabolism of the thyroidectomized rat. Sherwood, T. C., T. M. Wilson and H. Boneta, Am. J. Physiol. **120:**671. 1937.

Eighteen ovariectomized-thyroidectomized rats were injected with 1000 I.U. of amniotin daily for 3 days, without producing any change in the metabolic rate. It is concluded that amniotin has no measurable effect upon the basal metabolism of the thyroidectomized rat.—F. A. Hitchcock.

Microscopic studies of living thyroid follicles implanted in transparent chambers installed in the rabbit's ear. Williams, R. G., Am. J. Anat. **62**:1. 1937.

Thyroid follicles will survive indefinitely and can be studied microscopically for long periods by auto-transplantation into transparent chambers installed in the rabbit's ear. Transplanted follicles go through cycles the various phases of which are 1) secretion, 2) secretion and colloid release, 3) partial collapse, 4) recuperation. Variations in the shapes of grafted follicles are dependent on the stage of their activity. From this study it appears that the direction of secretion is toward the lumen, and it is suggested that the probable manner of colloid release is by diffusion across compressed cells chiefly in the second stage of the cycle. Observations are included on the reactions of follicles to extracts of the anterior pituitary gland, with brief consideration of nerves, lymphatics and blood vessels and their relation to the behavior of the grafts.—Author's Abst., Wistar Bib. Service.